

# MORPHOLOGY

FOUNDED BY C. D. WHITMAN

EDITED BY

J. S. KINGSLEY

Tulla College, Mass.

WITH THE CO-OPERATION OF

GARY N. CALKINS

Columbia University

Charles Otis Whitman  
1901

T. H. MONTGOMERY

University of Pennsylvania

W. M. WHEELER

Enlargement from a group photograph

Bussey Institution, Harvard University

WILLIAM PENTON

Dartmouth College

EDWIN G. CONNOR

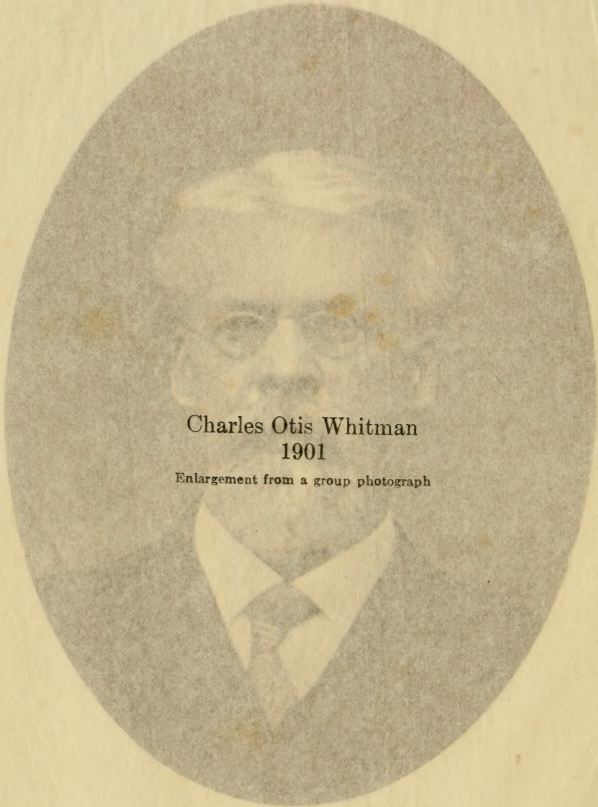
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VOLUME 23

1911

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

PHILADELPHIA



Charles Otis Whitman  
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Enlargement from a group photograph

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## PREFATORY NOTE

In 1909 a number of friends of Professor Charles Otis Whitman planned a volume of the *Journal of Morphology* as an acknowledgment of the debt of American science to him as the founder and editor of the *Journal*.

A committee, consisting of Frank R. Lillie, Edwin G. Conklin and Thomas H. Morgan, was appointed to receive contributions of articles from his former students and his associates of the Marine Biological Laboratory. It was decided that the numbers for the year 1911 should constitute a 'Whitman Volume.'

Professor Whitman died in 1910 before the first number was issued and the volume becomes a memorial to one who had a wide influence in the elevation of biological science.

As Whitman's ideals were broader than mere morphology, so the volume in the scope of its contents overlaps that field on all sides.

The articles which were accepted by the committee were so numerous and extensive that not all of them could be published during the present year, although the volume is far larger than usual; consequently some of them will appear, with proper acknowledgment, in the next volume of the *Journal of Morphology*.



THE  
CHARLES OTIS WHITMAN  
MEMORIAL VOLUME

EDITORIAL COMMITTEE

FRANK R. LILLIE  
EDWIN G. CONKLIN  
THOMAS H. MORGAN



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TO THE MEMORY OF  
CHARLES OTIS WHITMAN

1842-1910

THIS VOLUME IS DEDICATED

BY

HIS PUPILS AND ASSOCIATES

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# CONTENTS

1910

## No. 1. MARCH

BENNET M. ALLEN. The origin of the sex-cells of <i>Amia</i> and <i>Lepidosteus</i> . Twenty-seven figures.....	1
LEO LOEB. The cyclic changes in the ovary of the guinea pig.....	37
EDMUND B. WILSON. Studies on chromosomes. VII. A review of the chromosomes of <i>Nezara</i> ; with some more general considerations. Nine figures. One plate.....	71
C. B. DAVENPORT. The transplantation of ovaries in chickens.....	111
W. J. MOENKHAUS. The effects of inbreeding and selection on the fertility, vigor and sex ratio of <i>Drosophila ampelophila</i> .....	123
G. H. PARKER. The mechanism of locomotion in gastropods. One figure.....	155

## No. 2. JUNE

C. M. CHILD. The regulatory processes in organisms.....	171
LORANDE LOSS WOODRUFF. <i>Paramecium aurelia</i> and <i>Paramecium caudatum</i> . One figure.....	223
E. A. ANDREWS. Male organs for sperm-transfer in the crayfish, <i>Cambarus</i> <i>affinis</i> ; their structure and use. Four plates and thirty-one text figures..	239
WALLACE CRAIG. Oviposition induced by the male in pigeons.....	299
WILLIAM MORTON WHEELER. The ant-colony as an organism.....	307
GILMAN A. DREW. Sexual activities of the squid, <i>Loligo pealii</i> (Les). Four plates.....	327
FRANK R. LILLIE. Studies of fertilization in <i>Nereis</i> . I. The cortical changes in the egg. II. Partial fertilization. One double plate.....	361
W. E. RITTER and MYRTLE E. JOHNSON. The growth and differentiation of the chain of <i>Cyclosalpa affinis</i> (Chamisso). Four plates.....	395
OSCAR RIDDLE. On the formation, significance and chemistry of the white and yellow yolk of ova. Three plates.....	455

## No. 3. SEPTEMBER

CHARLES W. HARGITT. Some problems of coelenterate ontogeny. Three plates and three text figures.....	493
VICTOR E. SHELFORD. Physiological animal geography. Nineteen figures...	551
R. M. STRONG. On the olfactory organs and the sense of smell in birds. Two plates and four text figures.....	619
HENRY H. DONALDSON. On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog. Five charts...	663
RALPH S. LILLIE. The physiology of cell-division. IV. The action of salt solutions followed by hypertonic sea-water on unfertilized sea-urchin eggs and the rôle of membranes in mitosis. Three figures.....	695
THÓS. H. MONTGOMERY, JR. The spermatogenesis of an hemipteron, Euschistus. Five plates—147 figures.....	731
WINTERTON C. CURTIS. The life history of the <i>Scolex polymorphus</i> of the Woods Hole region. Thirteen figures.....	821

## No. 4. DECEMBER

H. H. NEWMAN and J. THOMAS PATTERSON. The limits of hereditary control in armadillo quadruplets: A study of blastogenic variation. Five figures and eight plates.....	855
CHARLES ZELENY. Experiments on the control of asymmetry in the development of the serpulid, <i>Hydroides dianthus</i> . Seven figures.....	927
WILLIAM A. LOCY. Anatomical illustration before Vesalius. Twenty-three figures.....	945
S. J. HOLMES. Minimal size reduction in planarians through successive regenerations.....	989
E. H. HARPER. The geotropism of <i>Paramoecium</i> . Five figures.....	993
ELLIOT ROWLAND DOWNING. The formation of the spermatophore in <i>Arenicola</i> and a theory of the alternation of generations in animals. Four plates and seven text figures.....	1001
Biography, Charles Otis Whitman. Five portraits.....	xv





# CHARLES OTIS WHITMAN

FRANK M. LEECH

Charles Otis Whitman was born December 14, 1857, at Woodstock, Vermont. He was educated in the common schools of his native town and in the University of Chicago, where he received his degree in 1881. He was descended from Jacob Whitman, a settler of Bridgewater, Massachusetts, who came to the town of Buckfield, Maine, in 1760. His grandfather, Joseph Whitman, was born in Woodstock, Maine, about 1780, and died in 1860. He was a member of the first century, among them Joseph (born 1780) and his son, Joseph (born 1810), grandfather of the subject of this sketch. Whitman's father (born February 18, 1810) was the fifth of ten children; his mother, Mary (born 1810) was the sixth of ten children.

Whitman was the youngest son of his father. He was educated in the common schools of his native town and in the University of Chicago, where he received his degree in 1881. He married Emily Nunn in 1887.

Whitman was a member of the first century, among them Joseph (born 1780) and his son, Joseph (born 1810), grandfather of the subject of this sketch. Whitman's father (born February 18, 1810) was the fifth of ten children; his mother, Mary (born 1810) was the sixth of ten children.

His early life was spent in Woodstock, Vermont. He moved to Waterford for a while, subsequently returning to Woodstock. He attended the common schools of Woodstock and Waterford "and fitted for college at Norway, Vermont, where he was teaching winters to obtain money for his college expenses." "He early developed a taste for natural history and while here (Woodstock) and at Norway he prepared a very fine collection of the birds of the State of Maine."

In the preparation of this leaflet, the following persons have been instrumental: Mrs. Whitman, Dr. Wallace C. C. Whitman, Prof. Cornelia M. Clapp, George T. Clapp, Mrs. Fletcher Day (Woodstock), Mrs. Helen Keith (Woodstock), Headmaster French (Woodstock), John Allis, Jr., Dr. Reuben (Woodstock), and others. The section dealing with the life of Whitman is based on the following sources: T. H. Morgan, E. G. Cook, Jr., J. P. Morgan, Jr., and others.



Charles Otis Whitman  
1857

Enlargement from a tin-type. Kindness of Professor E. S. Morse

## CHARLES OTIS WHITMAN

FRANK R. LILLIE

Charles Otis Whitman was born December 14, 1842, in Woodstock, Maine. He died at his home in Chicago, December 6, 1910. On his father's side<sup>1</sup> he was descended from Jacob Whitman, who was a resident of Bridgewater, Massachusetts, whence he emigrated to Buckfield, Maine. Three sons of Jacob Whitman settled in Woodstock, Maine, about the beginning of the nineteenth century, among them Joseph (born September 30, 1783), the grandfather of the subject of our sketch. Whitman's father (born February 19, 1821) was the eighth of ten children; his mother (born December 12, 1823) was Marcia Leonard, daughter of Solomon Leonard, also of Woodstock. Whitman married Emily Nunn of Peru, Ohio, in August, 1884, and had two sons, Francis, born in Milwaukee, Wisconsin, and Carroll, born in Worcester, Massachusetts.

His early life was spent in Woodstock, though his father removed to Waterford for a while, subsequently returning to Woodstock. He attended the town schools in Woodstock and Waterford "and fitted for college at Norway and other academies, teaching winters to obtain the means for paying his school expenses." "He early developed a taste for natural history, and while here (Woodstock) and a boy, he procured and mounted a very fine collection of the birds of Maine. So artistically pre-

<sup>1</sup> In the preparation of this brief biography I have been indebted for information to Mrs. Whitman, Dr. Wallace Craig, Prof. H. H. Donaldson, Prof. E. S. Morse, Prof. Cornelia M. Clapp, George T. Little, the librarian of Bowdoin College, Mrs. Cornelia Fletcher Day (Westford, Mass.), Mrs. Sarah H. Trumbull (Beverly, Mass.), Mrs. Helen Keith Frost (Westford, Mass.), Secretary of the Boston School Committee, Headmaster English High School, Boston, Mass., Prof. E. L. Mark, Edward Phelps Allis, Jr., Dr. Reinhardt Dohrn, and others. I must also express my indebtedness to E. G. Conklin and T. H. Morgan, for criticism of the manuscript. The section dealing with Whitman's scientific work was prepared by T. H. Morgan, E. G. Conk in, J. Percy Moore and others—See foot-note p. xlvii.

pared where they, and so naturally mounted, that they attracted much attention among ornithological students."<sup>2</sup>

It would be interesting to know more of his early life but Professor Whitman rarely spoke of it, though he referred at times to work on his father's farm. In reply to the question whether he had been interested in natural history as a boy, he replied to Professor Wallace Craig that he judged he must have been, because of his persistence in getting his grandfather to tell hunting stories. He never tired of the stories, and often walked a mile to have an evening of them; his grandfather was very kind in always telling these when asked. He also said that he kept pigeons as a boy, and was fascinated by them and sat and watched them by the hour, intensely interested in their feeding, their young, and in everything that they did.

We thus get a distinct though undetailed view of a boyhood spent on a New England farm, an education acquired by dint of labor and self sacrifice, and of an original interest in natural history, shown in his observation of pigeons and his collection of the birds of Maine.

He entered Bowdoin College as a sophomore in September, 1865, and graduated with the degree of Bachelor of Arts in July, 1868. The college curriculum of this time was the usual course of required studies with much emphasis on the classical languages, some study of modern languages and of mathematics, the elements of philosophy and a variety of sciences taught no doubt mainly from text-books. The influence of this classical education remained with him all his life, and was no doubt responsible for the views that he entertained in favor of the requirement of Latin for college education. There was certainly little to stimulate his interest in the field in which he subsequently won distinction. His membership in the Greek Letter Society Delta Kappa Epsilon, in the Athenaeum Society (literary), and in the Philologian Society (debating) may help to indicate his social and intellectual interests at this period. At his graduation he ranked about ninth in a class of twenty-three. The title of his commence-

<sup>2</sup> See Lapham, William B. "History of Woodstock, Me., with family sketches and an appendix." Portland, Stephen Barry, Printer, 1882.

ment oration "Free Enquiry" indicates already an unfettered mind.

On graduating from Bowdoin, Whitman was appointed principal of Westford Academy in Westford, Massachusetts. He began to teach there on December 16, 1868, and remained until the spring of 1872. He must have taught a great variety of subjects, to judge by the catalogue of 1872, as there was a four years' course involving mathematics, English, Latin, Greek, French, geography, book-keeping, history, natural philosophy, chemistry, mental philosophy, astronomy, physiology, and botany, and there were but two assistant teachers and ninety pupils in 1871-72. However, he continued his interest in birds and taxidermy, and the library of the Academy still has a good collection of Westford birds prepared by a lady whom Whitman instructed in the art while there; it also contains a fine specimen of one of the largest of Maine loons set up by Whitman himself.

During the school year 1871-72 Whitman substituted in the English High School in Boston, Massachusetts, and was regularly appointed sub-master in September, 1872. At that time the departmental system had not been introduced into the school and he taught general high school subjects. He remained with the school until the summer of 1875.

While in Boston Whitman came under the influence of Louis Agassiz, and was one of the fifty students who, in July and August, 1873, attended the Anderson School of Natural History founded by Agassiz on the island of Penikese. Here he met Professor E. S. Morse, who was an instructor under Agassiz, a circumstance which had a great effect in Whitman's later life, leading to his call to the University of Tokyo as related further on. Professor Morse was much attracted to him by the beautiful and accurate way in which he drew the lower forms of life, particularly the Ascidian *Perophora*, on which Morse himself was working at the same time. Morse and Whitman remained the best of friends throughout life, and at Whitman's invitation many years later Morse delivered several lectures at the Marine Biological Laboratory.

Louis Agassiz died in December, 1873, and the Penikese school was opened again in 1874 for the last time by his son, Alexander Agassiz. Whitman was again one of the privileged fifty who worked there, though ninety other applicants had to be refused admission for lack of accommodations. The Penikese school started a tide of biological work at the sea-shore in American which ebbed indeed for a while, but began to flow again in the decade of the eighties and has been running stronger ever since. No doubt the germ of the Marine Biological Laboratory, Whitman's most significant scientific enterprise, was implanted there in Whitman's heart and in the hearts of others. Doctor Craig states that Whitman felt that he got his first start in scientific zoology from Agassiz, but that he did not really get under way until he worked with Leuckart on *Clepsine* in Germany. Asked by Doctor Craig (in August, 1910) what he thought of Agassiz's method, Whitman replied that he did not think much of it at first but that as time went on he thought more and more of it. "We are apt to do the work for the student too much. What we should do is to set him a problem and let him work it out."

In 1875 Whitman decided to go to Germany to study natural history. Apparently he had not yet decided to abandon his career as teacher in the high school, for he left open the possibility of returning to his position after a year's absence. He sailed in July, 1875, and settled in Leipzig. From there he wrote to his successor in Westford Academy, Mr. William E. Frost, May 28, 1876: "Mr. Seaver (the head-master of the English High School at that time) says he will secure my re-election and another year's absence if possible. I have not much doubt of his ability to do this. At any rate I shall remain another year." But when 1877 arrived he was not yet ready to leave and he decided to remain a part, at least, of a third year. In 1878 he received the degree of doctor of philosophy from the University of Leipzig, and sailed for America in July of the same year, although he still wished "to remain a little longer in Deutschland, but the Fates say *no!*"<sup>3</sup>

<sup>3</sup> Letter to Mr. Frost, July 6, 1878.

In 1878 he published his first scientific paper "The Embryology of Clepsine" in the Quarterly Journal of Microscopical Science, vol. 18, pp. 215-315. This was his doctor's thesis; in many respects it was a very notable, indeed epoch-making work. It was the first time that the primordia of any ectodermal organs had been followed to individual cells, and that the cleavage process itself had been adequately interpreted as a process of 'histogenetic sundering.' He laid emphasis on the existence of embryonic axes in the unsegmented egg, and anticipated to a considerable extent views that did not receive adequate recognition until the period of study of 'cell-lineage' began about fifteen years later.

On his return to America he was appointed Junior Master, first grade, of the English High School in Boston, teaching English, and resigned in 1879. It is evident that now for the first time, at the age of nearly thirty-seven, he had irrevocably decided to devote himself entirely to zoology, for by his resignation he burned his bridges behind him. He received an appointment as fellow in biology in Johns Hopkins University for 1879-80, but he did not enter on the fellowship, having in the meantime accepted the chair of zoology in the University of Tokyo. He sailed for Yokohama, August 21, 1879. On the voyage he made observations on the flight of flying fish which he described in the American Naturalist, vol. 14, 1880, maintaining that their course through the air is actual flight.

#### WHITMAN IN TOKYO

With his appointment to the University of Tokyo in 1879, begins Whitman's real influence as a teacher and organizer in zoology. He was then nearly thirty-seven years of age and had passed through a most varied preparation for his life-work. He had pushed on without haste but without rest, always carrying with him his original and vital interest in living things since he first studied pigeons as a boy, in spite of the necessity of earning a livelihood by teaching school. He thus came to his chosen life work in full maturity with a mind broadened by varied experiences, yet with actual boyish enthusiasm and interest, that never left him throughout life.

The work in zoology in the Imperial University of Tokyo was first organized by Professor E. S. Morse, who was invited from abroad in 1877.<sup>4</sup> He remained there two years and was succeeded in 1879 by Professor Whitman. Professor Iwakawa states that Professor Huxley was first invited by Professor Morse to accept the chair as his successor. Professor Huxley wrote that for years he had been desirous of studying biology in oriental countries and that the present call from Tokyo was the best chance he could ever have; however, he regretted that the declining condition of his health would not allow him to accept. Professor Morse states in a letter that while instructor at Penikese he had known Whitman and was much impressed by the beauty and accuracy of his work; his experience as teacher in Boston was also a recommendation; so Professor Morse secured Whitman's call to the chair of zoology in Tokyo and it was accepted. Professor Whitman remained in Japan for two years until 1881. He had only four students, but as all became professors of zoology in the Imperial University he may be justly regarded (as Dr. Takahashi states) as the father of zoology in Japan. Professor Iwakawa says that Professor Whitman's teaching really laid the foundation of modern zoology in Japan.

It is impossible to reproduce the tone of affection and reverence in which these reminiscences are written by two of his original pupils, Iwakawa and Ishikawa, and a later student during the Chicago period, Takahashi. Professor Iwakawa says, "Once he was my teacher while he was in Japan and since then until today I have been paying respects and admiration both for his character and for his work in biology." "I am constrained by what I regard as a duty to him to let others get a glimpse of what I knew him to be while he was with us in Tokyo"—and the whole tenor of his reminiscences is one of affectionate admiration and devotion. "Professor Whitman's attitude of mind toward his

<sup>4</sup> In the *Magazine of Zoology*, published by the Zoological Society of Japan, Tokyo, vol. 23, no. 269, March 15, 1911, there appear three articles on Professor Whitman, the first by Professor Tomotaro Iwakawa, the second by Professor Chiyomatsu Ishikawa, and the third by Dr. Katashi Takahashi. For the translation of these articles I am indebted to Dr. Shigeo Yamanouchi. They form the basis of the following account.



Charles Otis Whitman

1882

From Lapham's History of Woodstock, Maine.



pupils was such as a mother toward her son." Professor Ishikawa writes in a similar spirit throughout. He says, "On receiving the tidings of Professor Whitman's death I am very much surprised and bitterly mourned." "I mourned bitterly in the recollection that those delightful days we had together shall never again be realized, but have now become a memory." "The work he has done during his life still remains and will be remembered forever." Takashahi says, "As he was the teacher of our professors, he will be justly regarded as our father of zoology in Japan. I feel as if I had lost my grandfather because of his being the teacher of our professors and because of his cherished kindness shown to me as a father might have shown to his son during my stay in his laboratory in the University of Chicago."

The following incident, as related by Professor Iwakawa and translated by a Japanese friend, is worth quoting:

For the purpose of making bird specimens for the museum, the University secured two government licenses in hunting seasons and the licenses were handed to the Zoological Department for the use of the students.

To make the specimens was one of purposes of hunting and the other end seemed to eat flesh of birds. One Saturday, a number of pigeons was brought to our laboratory and the next day being Sunday, some of us came to the laboratory to have the share of feast. Dr. Iijima dissected the birds. A fire shovel was cleansed and put lard on and then flesh; then put into the stove to fry. Salt, sauce, knife and fork were ready and the party waited to have the flesh cooked. Dr. Sasaki had belatedly come. Being he was an hearty eater, the party who were already there refused to add him into the company and so all went to Dr. Whitman's office and locked the door from inside so that he could not get in. Unexpectedly Dr. Whitman came, in spite of that the day was Sunday. He put his slippers on as usual and tried to get in his office by the door he used to enter. To his surprise the door was locked. He came over to our laboratory and there Dr. Sasaki sat alone. Professor Whitman tried to open the door that leads to his office from our laboratory. Again to his surprise the door was also locked. Dr. Sasaki being left very much uneasy, called out, 'Professor Whitman has come!' The party inside the door, including Dr. Iijima, believing that Professor Whitman would never come on Sunday and that the warning might be Dr. Sasaki's stratagem to induce the party to open the door, took the alarm easy and were chattering over quite noisily. Then there was heard a voice from outside, 'Who are in the room?' Evidently that was Whitman's. Frightened all at once the party fled

to a court from the door which leads to the corridor. The party now in the court, sent a spy to look after what Professor Whitman was doing and assured that he, taking a few things, has gone home. As the coast was clear, the party returned to the laboratory. Dr. Sasaki who had been left alone in the laboratory, had already helped himself alone with the fried birds that were left in the stove, and was sitting quite satisfied. He spoke smilingly to the party, when they entered in. 'I was very much embarrassed indeed! The handle of the shovel was peeping outside the mouth of the stove. Fried birds were making cooking noise inside and a tempting odor was ejecting from within. But our teacher had not asked a bit. Simply he said, 'who are that ran out of the room?' So I answered, 'I do not know.' 'At any event I thank you for your kind feast.' Dr. Iijima seemed very much disappointed; as yet the case being as such that Dr. Sasaki only cannot be blamed at, and he spoke to Dr. Sasaki, 'You lucky fellow.' The following day we were in a constant concern and reluctantly expected some sort of punishment on our conduct of the previous day. On the contrary Professor Whitman didn't even ask a word about what thus happened the previous day. Professor's Whitman's attitude of mind toward his pupils was such as mother toward her son.

There were but two rooms devoted to the department of zoology in the Imperial University in Whitman's day; literature and apparatus were very scanty and Whitman first introduced modern laboratory equipment and methods in microscopical technique. His four students were Ishikawa in the first year class, Iijima and Iwakawa in the second year, and Sasaki in the third year. There was an assistant, a janitor and two artists, all of whom were kept busy collecting and drawing leeches. It was characteristic of Whitman that he should set each of his four students to work on a special problem for research, even Ishikawa in his first year of zoology.

Hard work was the order of the day in Whitman's laboratory; he set the example himself, and the students, who lived in a dormitory near by, often worked until midnight. Twice a day Whitman consulted with each student about his work. In the absence of a University biological library Whitman placed his own journals and books at the disposal of the students and aided them in translating German and French. He kept each man close to the study of his individual problem and deplored the wasting of time spent on other subjects. From time to time he delivered lectures on special topics and as a general course he expounded Spencer's Principles of Biology.

At the end of two years each of the four students had a paper ready for publication and Professor Whitman presented them to the Journal of the College of Science of the Imperial University for publication; the officials in charge of the journal replied that, as the journal was organized for the purpose of publishing the researches of professors, any theses of students worth publishing should be published under the name of the professor. This aroused Professor Whitman's indignation, and he withdrew the papers, remarking that he would never again present papers to the University for publication. He then sent three of them to the Quarterly Journal of Microscopical Science where they were published. Sasaki's paper on Salamander was published after Whitman's departure in the Journal of the Science College.

This incident seems to have been the beginning of an estrangement between Whitman and the administration of the University, which was aggravated by the inability or unwillingness of the administration to accede to many of his requests for more adequate equipment for the department. The period of his appointment having come to an end in 1881, the University requested him to remain, but the proposal was refused and in August, 1881, he left Japan without bidding formal farewell to the University. He published a short brochure entitled "Zoology in the University of Tokyo" shortly before he left, but that he had no intention of wantonly hurting Japanese susceptibilities is evident, as Professor Ishikawa states, from a thorough study of it. He had made a close study of the Japanese and he discovered and pointed out their most obvious weak points in an honest and essentially friendly fashion. "Professor Whitman loved Japan and sympathized with the Japanese. That his love and sympathy poured forth to the Japanese in a degree far surpassing any ever shown to us was marvelously evidenced at the time of the Russo-Japanese War" (Ishikawa). Indeed, those of us who were with Whitman at this time knew that he could not have suffered more keenly in the misfortunes or rejoiced more in the triumphs of his own country. This was fully realized by the Japanese people and the slight unpleasantness of his departure was soon forgiven.

## IN EUROPE

Professor Whitman left Japan in August, 1881, and from November 11, 1881, to May 2, 1882, he worked at the Zoological Station of Naples as guest of Professor Dohrn. His sojourn in the Zoological Station laid the foundation of an everlasting friendship with Dohrn, and, when he left, Professor Dohrn gave him a testimonial recommending him strongly to some professorship. While at Naples Whitman studied the embryology, life-history, and classification of Dicyemids and wrote a paper on the subject, published in January, 1893, which is still the standard work of reference.

Whitman had thus come under the influence of three of the great leaders of his time in zoology, Agassiz, Leuckart and Dohrn. His original bent in the direction of the natural history of birds was diverted by these experiences towards the study of marine life and lower organisms, but later on he returned to his original interests in birds, particularly pigeons, with a mind deepened by intimate acquaintance with the fundamental problems of biology.

After leaving Naples he went to Leipzig where he remained until the middle of September, engaged among other things in preparing his Naples work for publication. On September 1, 1882, he wrote to Mr. Frost: "I leave for America on the 15th of September and shall go to Leonard's (Newton Highlands) and shall hope to see you somehow or somewhere. I have not yet decided where to spend next winter. There is some possibility of my going to Johns Hopkins—though nothing definite yet. Have just finished manuscript of work done in Naples, and a portion is already in print."

## AT HARVARD

In the autumn of 1882 he was appointed Assistant in Zoology at the Museum of Comparative Zoology of Harvard University, and held this position until 1886. In the spring of 1883 he went to Key West, Florida, to secure for Mr. Alexander Agassiz material with which to complete Mr. Agassiz's monograph on "The Porpitidae and Velellidae." Though he spent six weeks there he did not meet with success. In the summer of 1883 he worked at

Mr. Agassiz's Newport Laboratory on the development of pelagic fish eggs. Some of the results, worked up later in Cambridge, were published with Mr. Agassiz in two papers (1884 and 1889). In the first paper the origin of the periblast was correctly described for the first time, a most important contribution in view of the confusion of opinions on this subject. During this summer he met his future wife, Miss Emily Nunn, who was also working at Mr. Agassiz's laboratory.

The years 1883-1886 were productive years; during part of this time Whitman edited the department of "Microscopy" of the *American Naturalist*. He worked out and published his papers on "A Rare Form of the Blastoderm of the Chick" ('83), "External Morphology of the Leech" ('84), "On the Development of Some Pelagic Fish Eggs" ('84), "Segmental Sense-Organs of the Leech" ('84), "The Leeches of Japan" ('86), "The Germ-Layers of Clepsine" ('86), and some minor papers. He also prepared and published his book on "Methods of Research in Microscopical Anatomy and Embryology" ('85) (see Bibliography).

#### THE LAKE LABORATORY AND THE FOUNDING OF THE JOURNAL OF MORPHOLOGY

From 1886 to 1889 Whitman acted as director of the Lake Laboratory at Milwaukee, Wisconsin,<sup>5</sup> founded by Edward Phelps Allis, Jr. Mr. Allis had decided to start a laboratory for biological and related research and Whitman was recommended to him as a proper person to take charge. There followed a conference in which the plans and purposes of the laboratory were discussed, and Whitman then presented the need of an American journal for publication of zoological research, pointing out that American workers were obliged either to present their papers to some scientific society or to send them for publication to some one of several European journals. Whitman then asked Mr. Allis if he would consider the publication of such a journal, in connection with the laboratory. He was asked to submit figures and

<sup>5</sup> I am indebted to Mr. Allis for some of the information on which the following statements are based.

plans, and it was finally arranged that he should come to Milwaukee, take charge of the laboratory, to be known as the Lake Laboratory, and also edit with the cooperation of Mr. Allis, a journal to be called the *Journal of Morphology*. The journal was to be a model of publications of the kind.

Whitman may not have been the first to realize the need of establishing a journal of zoological and anatomical science in America, but he was the first to possess sufficient courage, energy and influence to set about realizing the need. He was fortunate indeed to find a man of scientific attainments and enthusiasm with an ample and liberal purse to support him in this undertaking. In the introduction to the *Journal* Whitman wrote, "The mixed character and scattered sources of our publications are twin evils that have become intolerable both at home and abroad. The establishment of the *Journal of Morphology* may not be the death blow to these evils; but there is hope that it will, at least, relieve the more embarrassing difficulties of the present situation."

In its make-up both scientific and typographical, the *Journal of Morphology* was a model of what a research publication should be, and it did much to coordinate zoological research in America, to give it a worthy setting, and to make it better known abroad. Eighteen volumes were published between 1887 and 1903, always at considerable financial loss, and its publication was then suspended for a while in spite of Whitman's efforts to secure the needed support. The *American Journal of Anatomy* and The *Journal of Experimental Zoology*, begun in the period of suspension of the *Journal of Morphology*, did not, however, suffice for the growing needs of zoological and anatomical science, and the *Journal of Morphology* was taken up again by The Wistar Institute of Anatomy and Biology in Philadelphia, in 1908, and its publication has continued ever since. As Professor Mall says, "The *Journal of Morphology* served as a model for many of our scientific journals, both biological and medical, which have come into existence during the past twenty-one years. The importance of sound scientific journals to anatomical and zoological science is now clear to all, and both anatomists and zoologists

owe to Professor Whitman a debt of gratitude for having been the pioneer in this field" (*Anatomical Record*, vol. 2, 1908, p. 381).

In 1898, realizing the need of some means for more rapid publication than was afforded by the *Journal of Morphology*, Whitman started the *Zoological Bulletin* with the cooperation of W. M. Wheeler. The idea was to afford means for the rapid publication of shorter articles and preliminary notices dealing with investigations in zoology which required only simple illustrations. The *Bulletin* was therefore published monthly. It was intended to be a companion serial to the *Journal of Morphology*. After the publication of two volumes the name was changed to the *Biological Bulletin* and it was transferred to the Marine Biological Laboratory as its official publication.

At the Lake Laboratory Whitman was associated with Edward Phelps Allis, the founder, Howard Ayers, William Patten, A. C. Eycleshymer, and some others. The work of the laboratory was research work in morphology, especially embryology. Whitman himself began investigations on *Amia* and *Necturus*, but though he carried some of this work quite far, but little of it was ever published. His scientific activity during this time may be inferred from the list of publications covering the period 1886 to 1889.

#### AT CLARK UNIVERSITY: 1889-1892

In 1889 Whitman accepted a call to the chair of zoology in the newly founded Clark University of Worcester, Massachusetts. Professor G. Stanley Hall of Johns Hopkins University had sought to establish with the aid of Jonas Clark of Worcester, a strictly graduate and research institution, which should accomplish all that the Johns Hopkins University had set out to do in elevating the standard of scholarship in America, but without the hindrance of undergraduate instruction. Whitman met there with thoroughly congenial conditions and associates. President Hall had assembled a small but remarkable group of scientific men, all animated by the same high ideals of scholarship. They were unencumbered with undergraduate instruction, provided with fairly adequate means for research, and they seemed destined to realize the fine aim that President Hall had set before them.

Whitman's teaching career, interrupted since he left Tokyo eight years before, was now resumed, and continued to the time of his death. A small body of research students was attracted to him, who carried on their work in Worcester during the academic year and at the Marine Biological Laboratory in Woods Hole during the summer. Whitman's laboratory was a paradise to the properly qualified research worker. There was practically no set instruction and the student's liberty was complete in all respects, but a spirit of hard work and complete absorption in the fundamental problems of biology prevailed. The problems of biology were the true topics of the day and, when the zoological club met, such subjects as Darwinism and Lamarckism were discussed with a fire and enthusiasm comparable to the most intense political or religious controversies. The main business of each student was his research problem, a secondary business was the preparation of some subject set for presentation at the zoological club, and the animated discussion of fundamental problems of biology prevented too much narrowness. Students read much and thought much because they had both time and inclination, and were not subject to trivial academic demands.

Whitman had a great respect for the intellectual independence of his students. He set them worthy problems but left the working out to the student; he was at the same time their severest and most friendly critic. He maintained their courage through difficulties, rejoiced with them in their discoveries, and always acknowledged their complete ownership in their results. He required convincing proof of each statement, and one could feel sure that whatever passed him would stand. He was completely loyal to them in all relations, and it is characteristic that the main event which finally induced him to resign and move to Chicago was an act of the administration which he regarded as an injustice to one of his students. He was not alone in his displeasure with the administration, though the causes were various and the departments of physics and chemistry, zoology, anatomy, neurology, and palaeontology of the new University of Chicago were organized by seceders from Clark University in 1892.

## PROFESSOR WHITMAN AND THE MARINE BIOLOGICAL LABORATORY

The organization of the Marine Biological Laboratory was a response to the same demand that established and maintained a marine laboratory on the island of Penikese in 1873 and 1874. In his address at the opening of the Marine Biological Laboratory Professor Whitman said:

The Annisquam Laboratory, the immediate predecessor of this, was organized to serve the same ends as the Penikese School, and the forces there engaged have simply been supplemented and transferred to the new Marine Biological Laboratory of Woods Hole, with such changes only as circumstances have rendered necessary. It was through the generous support and active cooperation of the Woman's Education Association of Boston that Professor Hyatt was able to maintain the Laboratory at Annisquam, and the same Association initiated and carried through the movement that has given us this Laboratory.

In 1886 efforts were made by the Association to place the Annisquam Laboratory on an independent and broader foundation. A circular letter sent to many of the leading biologists of the country received encouraging replies and accordingly a preliminary meeting was held on March 5, 1887, in the library of the Boston Society of Natural History. A committee was there organized to perfect plans for the organization of a permanent sea-side laboratory, to elect trustees and to devise ways and means for collecting the necessary funds. The committee met with sufficient success for a modest beginning and accordingly in March, 1888, the Marine Biological Laboratory was formally incorporated with ten members. Seven trustees were chosen at a meeting of the Corporation held the same month. In June, 1888, the Trustees issued a circular in which they announced the policy of the Laboratory to support instruction as well as research, and invited the cooperation of the universities and colleges of the country. Professor Whitman's appointment as director of the Laboratory was also announced in this circular.

This brief account of some facts in the early history of the Marine Biological Laboratory may suffice to show the origin of Professor Whitman's connection with the institution. He found a local organization that planned to become national in scope,

to enlist the cooperation of colleges and universities throughout the country and to provide for research and instruction in biology. The location of the Laboratory was also fixed and the first building erected at Woods Hole. Although the incorporators were all residents of Boston, yet they had provided for a national organization by offering each institution invited to cooperate the privilege of naming five members each of the Corporation during the term of cooperation. Apparently, Professor Whitman had nothing to do with the original statement of these principles, but after his appointment as director, at least, he became their chief exponent and developed them to a much greater extent than the original incorporators had intended, so that the Corporation soon came to have a large and nation-wide membership, and the Board of Trustees was enlarged to include 12 members in 1890, 17 in 1892, and 21 in 1895. The membership of the Corporation grew by leaps and bounds, and rapidly became representative of the entire country, as the practice was followed for some years of inviting all who worked at the Laboratory to become members. The attendance at the Laboratory was 17 in 1888, 44 in 1889, 47 in 1890, 71 in 1891, 110 in 1892, 199 in 1895; and the number of institutions represented was 13 in 1888, 29 in 1889, 32 in 1890, 31 in 1891, 52 in 1892 and 85 in 1895.

The early years of the Laboratory were years of great prosperity; to accommodate the growing tide of workers an L was added to the original building in 1890; in 1892 a building equal to the original Laboratory in size was added to form the third side of a quadrangle, and two separate buildings, one for botany and another for a lecture hall and research rooms were added by 1896.

Whitman's part during this period of rapid material development was to furnish the spirit and develop the ideals of the institution. It is obvious that the idea of cooperation had a primary practical significance in the minds of the original trustees, to secure support for the new institution. Though he did not lose sight of its practical significance, the idea of cooperation was transformed by Whitman into an ideal of a scientific democracy, which furnished a motive for loyalty and devotion such as rarely, if ever, existed in a scientific enterprise, so that the development

of the Laboratory became a kind of cult to a large and influential body of naturalists. Whitman not only awakened this spirit, which was compounded of devotion to himself as well as to the ideal which he represented, but he kept it alive, and more than once, by refusing to compromise any fraction of the fundamental idea for immediate practical advantage, he saved the principle from extinction. That the Laboratory today is still a scientific democracy is due entirely to Whitman's uncompromising devotion.

In his first report Professor Whitman states, "The new Laboratory at Woods Holl is nothing more, and, I trust, nothing less, than a first step towards the establishment of an ideal biological station, organized on a basis broad enough to represent all important features of the several types of laboratories hitherto known in Europe and America." Thus he formed great plans for the germinal institution. He early maintained that in such an ideal biological station it was essential that all biological interests should be represented, and accordingly successively added departments of botany, physiology and embryology to the original zoology, each with its side of research as well as instruction. But the variety of work that has been welcomed at Woods Hole cannot be included even within these broad divisions. Professor Whitman had most catholic interests in biology and it is remarkable in what fundamental ways he comprehended the problems of each division. The association of workers in different fields of biology has been one of the most helpful and stimulating features of the Station.

The Marine Biological Laboratory was designed for instruction as well as research. The original circular opens with these words: "The Trustees of the Marine Biological Laboratory earnestly desire to enlist your cooperation in the support of a sea-side laboratory for instruction and investigation in biology." Instruction was in fact placed first, not only in the opening sentence but throughout the circular. However, the Laboratory started out at once under Whitman as primarily a research institution, and in his address at the opening of the Laboratory, July 17, 1888, he said:

In every attempt hitherto made to combine the two chief interests here represented, instruction has been the object of first concern. Now the only way to keep the distributive function efficient and active is to unite it in proper relations with the productive function. The Laboratory (i.e., the side of investigation) is the creative agent—the source of all supplies; the school is merely the receiver and distributor. Any attempt to combine the two which ignores or reverses these relations must end in disappointment and failure.

In the fifth annual report Professor Whitman states:

The two functions of instruction and investigation have worked admirably together, each growing stronger in the success of the other. We have endeavored to keep the two properly balanced, but I think we have nearly reached the limit of our capacity for instruction with our present space and means. We already see that to tax our teaching force much more would not tend to improve the side of investigation.

In the eighth annual report for the year 1895 Professor Whitman again returns to this theme:

Our instruction and investigation have been inspired by a common purpose, and thus kept in such relations that each has added to the strength of the other, and added more and more with every stride forward. If instruction has increased, it is chiefly due to the stimulating influence of investigation; if investigation has gained, it is because instruction has multiplied workers. Mutual service is the bond of union, but the union is not merely one of coordination, in which the two elements are simply balanced one against the other; it is one of a more vital order, in which each is servant and only one is master. All our classes face in one direction—towards original work—and all our activities, sympathies and interests are dominated by the spirit of research. Does that render our instruction less efficient? Just the contrary. It fills with life and purpose, makes students more earnest, dignifies the work of the teachers, and wins their best effort. Moreover, it re-enforces the service of the regular staff by contributions from every member of the investigating departments.

Farther on:

What does instruction mean for us? It means, not wholly, but pre-eminently, preparation for original work, and much of it is especially designed for the benefit of investigators, not beginners only, but for specialists who are independent workers.

It will be plain, I trust, that we are not cultivating two antagonistic functions, between which we have to carefully guard the balance, lest one may prosper at the expense of the other. There can be no excess in either direction, for every gain, whether on one side or the other, is a gain not only for the part but also for the whole.

These extracts explain Whitman's position with reference to the functions of instruction in a primarily research institution. His ideas seem to have been sound, if we may judge from the experience of twenty-three years, during which the two have existed side by side with mutual advantage.

During the third session of the Laboratory Whitman organized the evening course of Biological Lectures which has proved ever since one of the stimulating features of the Laboratory life. In his report for this session Whitman outlines the idea as follows:

These were not intended to take the place of systematic lectures, such as are given in the regular courses of instruction; they stand rather for the higher and the more general needs of the science. Their leading purpose, if I may be permitted to define it more with reference to the possibilities of its future development than to its present attainment, was to meet the rapidly growing need of cooperative union among specialists. Specialization has now reached a point where such union appears to be an essential means of progress. Specialization is not science, but merely the method of science. For the sake of greater concentration of effort, we divide the labor; but this division of labor leads to interdependence among the laborers, and makes social coordination more and more essential. This is the law of progress throughout the social as well as the organic world. An organism travels towards its most perfect state in proportion as its component cell-individuals reach the limit of specialization, and form a whole of mutually dependent parts. Scientific organization obeys the same law. As methods of investigation improve, specialization advances, and at the same time the mutual dependence of specialists increases. Isolation in work becomes more and more unendurable. Comparison of results, interchange of views and ideas, and a thousand other advantages of social contact, become of paramount importance to the highest development.

In such considerations may be found the leading motive for this course of lectures. While directed in the main to the higher needs of investigators, they deal, as a rule, with subjects of present and quite general interest to beginners. In general, it may be said that the authors undertake to set forth what has been accomplished in their special fields of research, to give the conclusions of the best work and thought, to point out general bearings, and to state the problems that await solution.

The educational value which such lectures may be presumed to have, and the consideration that through them the aims, the needs, and the possibilities of biological work might, in some measure, be made better known to the public, especially to those whose liberal benefactions have enabled the Laboratory to carry forward its work, suggested the propriety of publication.

At various times these lectures, which have sometimes taken on a spirit of some formality, have been supplemented by informal discussions following lectures delivered by investigators before classes, especially the class in embryology during the early years, and later in physiology; at other times research seminars have been formed for the distinct purpose of discussing and criticising work presented by the investigators; and at all times in the history of the Laboratory free and informal discussion between investigators of their work in progress has been a characteristic feature in the laboratory life. In all this the steady and sane influence of Whitman was at work. All coveted discussions with Whitman; he had a most sympathetic interest in all work going on in the Laboratory, and deep insight into the fundamental problems. One frequently discovered after unburdening one's self in response to his sympathetic attitude that he had thought out the problem in question more thoroughly. But his courteous and honest attitude always saved such a situation from being painful. He exercised in these ways a steadying influence on the investigations of others, for he was never hurried into following a mere fashion in research.

The social life of the Laboratory in Whitman's time was simple and sincere. He had a horror of all formality and met everybody on a plain and equal footing. His hospitality usually took the form of small dinners particularly well cooked and served, with not more than half a dozen guests usually. He was a most charming host, gracious and self-effacing. The conversation usually turned on some scientific subject and he had the knack of making the others talk, and it was considered quite a triumph for the others to draw him out. He sustained relations with his students both at Woods Hole and elsewhere, that can only be described as fatherly. He often helped them financially, and stood by them with the greatest loyalty in securing positions. To the respect that all his students felt for his scholarship and ability was added the love and devotion that they owed to the best of friends.

No account of Whitman's relations to the Marine Biological Laboratory would be complete which failed to describe his con-

duct in various crises of the history of the institution. The essential character of the man comes out better probably in its mingled elements than in any other known relations. But this account must necessarily be incomplete and partial to the extent that Whitman is the subject, and not the Laboratory. Up to about 1895 the relations of the Director and trustees seem to have been on the whole cordial, in spite of minor difficulties. But the rapid growth of the Laboratory imposed financial burdens of no slight amount. In 1890 an 'L' was added to the original building; in 1890 a new wing was built; in 1893-4 a new dining hall and kitchen were erected, and the present botanical laboratory. The expenses of these additions was met by numerous contributions from friends and by a loan of \$3,500 secured by a mortgage upon the property of the Laboratory, and an unsecured loan of \$3,000 from one of the trustees.

The Boston trustees themselves felt great satisfaction in the rapid growth of the Laboratory. In 1894 they could say: "The only serious perplexities of the last year have been the result of its rapid growth and prosperity;" the Laboratory had in fact become self supporting so far as current expenses were concerned. It was important, however, to meet the outstanding loans for new buildings and the following appeal was issued:

Reluctant as the trustees were to incur expenses which would make it necessary, in this time of financial stress, to ask help from the friends of the Laboratory, yet, in the opinion of many, to have checked the growth of the institution at this stage, by turning away desirable students and investigators, would have inflicted a permanent injury. We ask, then, from those whose conviction of the value of such a Laboratory has helped to bring it to its present condition of prosperity, still further aid in its future development (from the Trustees' Report to the Corporation for the year 1894).

But the enlargements, great as they had been, were still inadequate to the growing demand. In proposing the further enlargement which Professor Whitman felt to be necessary to provide for the growth of the Laboratory, he was hampered by the reluctance of some, at least, of the trustees, to incur further indebtedness. A new building was needed of the size of the original laboratory to provide a lecture hall and more rooms for inves-

tigators at an estimated cost of \$3,000. Professor Whitman organized the investigators of the Laboratory into a Biological Association to work for the needed building. This Association pledged \$1,500 towards the cost of the new building, and the trustees finally agreed to secure an equal sum. The building was erected in 1896, and has been fully occupied ever since, thus justifying Whitman's estimate of the needs of the Laboratory. But this plan left the debt for previous buildings still outstanding. "Subsequent events showed that Doctor Whitman raised the whole of the \$3,000, besides the money needed for equipment, and the trustees did not as a *body* raise anything; although a few individuals who were supporters of Doctor Whitman and his policy raised a few hundred dollars" (from "A Reply to the Statement of the Former Trustees of the Marine Biological Laboratory," 1897, p. 8).

While it is perhaps undesirable to revive old controversies, yet it seems needful in justice to Doctor Whitman, to state the issues of the years 1896-1897, with the dispassionateness which fourteen elapsed years should furnish. It was never true that a majority of the board of trustees lost confidence in, or were out of sympathy with Doctor Whitman; but a minority of the board, who nevertheless constituted the governing element by virtue of their original membership and residence in Boston where all the meetings were held, were much displeased with him for not listening respectfully enough to their motives of caution, and for his dominance in Laboratory affairs. The existence of a small deficit in the operating expenses of the year 1896 led them to declare that the Laboratory should not be opened in 1897, unless a sum of \$2,000 were raised not later than April 15. This sum was much in excess of the deficit and the vote was not taken until February 5, 1897. An offer on the part of one of the trustees, Mr. L. L. Nunn, to bear any added deficit resulting from operations of 1897, was refused. The trustees raised the sum of \$1,140 by April 12, and the treasurer reported on May 5 that there was a balance in the treasury of \$735.55; there was also about \$670 accumulated interest in funds available for any purpose the trustees might approve. The deficit in the meantime had melted away. The



October 10, 1908. Photograph by R. M. Strong



1908. Photograph by Kenji Toda

Charles Otis Whitman



announcement of the 1897 session was therefore very late and the attendance suffered seriously in consequence of the rumor that had spread that the Laboratory would not be opened that year.

A meeting of the board of trustees was held at Woods Hole on August 6, 1897, and at this meeting a majority of the members present, who were favorable to Whitman, voted to call a special meeting of the members of the Corporation to be held in Boston on August 16 for the purpose of considering changes in the by-laws. The purpose of the proposed changes was (1) to provide that the annual meeting of the Corporation should be held in Woods Hole instead of in Boston, and in August instead of November, and to increase the quorum so as to secure a more representative attendance and avoid local control, and (2) to change the body of the trustees from a body practically self-perpetuating to an elective body, elected by the Corporation in four groups, one such group to be elected each year for a period of four years, and thus avoid the old practice of the simultaneous annual election of all members.

At this meeting about eighty-seven members of the Corporation recorded their names with the clerk, and it was estimated that there were about twenty others present who did not do so. It was the largest and most representative meeting of the Corporation ever held up to that time. The program as outlined was unanimously adopted.

This amounted to no less than a revolution in the government of the Laboratory, and the action was promptly followed by the resignation of seven out of the nine members of the board of trustees resident in Boston and its vicinity. Six of these and one other trustee then drew up a statement which was primarily an attack on the Director, Professor Whitman, which they published in *Science*, October 8, 1897. To this statement a complete reply was made in the more dignified, but less permanent, form of a separate pamphlet by a committee of three of the trustees who stood by the Director ("A Reply to the Statement of the Former Trustees of the Marine Biological Laboratory," Boston, Alfred Mudge and Son, Printers, No. 24 Franklin Street, 1897.) This reply and the facts that two-thirds of the board of trustees stood

by Professor Whitman, that the places of the 'former trustees' were taken by well-known naturalists, and that the progress of the Laboratory was not seriously interrupted even by so serious a controversy, constitute a sufficient vindication for Whitman.

This struggle was unfortunately necessary to establish the national, representative and democratic character of the institution, a character that grows with the years and which commands the loyalty and devotion of the present members, both of the Corporation and of the board of trustees.

Once again it was necessary for Whitman to take a firm stand to maintain the fundamental ideals of organization of the Laboratory. This was when the newly organized Carnegie Institution of Washington offered in 1902 to take the Laboratory as a department. This would have permanently solved the difficult problem of maintenance, but Whitman was convinced that it would destroy the representative democratic character of the institution, although every possible concession to the existing form of organization was generously offered by the Carnegie Institution. In this opinion he stood nearly alone, but none the less firmly, and it was his insistence that finally brought about a delay of the decision with an annual grant of \$10,000 a year for a period of three years (1903-1905) from the Carnegie Institution in the form of a subscription to twenty work rooms. At the end of this period a very notable petition signed not only by all members of the laboratory, but also by a large number of representative naturalists, for the continuation of the temporary arrangement was not granted by the trustees of the Carnegie Institution, and the original proposal lapsed. The independence of the Laboratory had been maintained, but it was apparently as far from a stable basis of financial support as ever.

Following this, Whitman gradually withdrew from active participation in the management of the Laboratory, although he retained the title of Director until 1908. However, he no longer attended meetings, and was even absent from the Laboratory for two successive seasons, 1904 and 1905. The house which he had occupied at Woods Hole burned down in the winter of 1905-1906; and, fearing that this would make his return impossible

his friends raised a sum of \$3,000 by subscription and the property was bought and the house restored and presented to Whitman. This very signal mark of love and appreciation on the part of his friends, indicating as it did so clearly their desire to remove every obstacle that prevented his presence among them, touched Whitman most deeply. He was present again at the Laboratory in the sessions of 1906 and 1907, but never again, except for a brief visit of two or three days in 1909.

His gradually increasing engrossment in the study of heredity and evolution in pigeons may be assigned as the principal cause of his withdrawal from residence at the Laboratory. For many years he transferred his large collection of birds from Chicago to Woods Hole and back again each summer. He always suffered some losses of valuable birds, even when the railroad companies allowed him to take his birds as excess baggage and to attend to them en route. However, when this permission was refused and they had to come by express and might be delayed over an extra night, the losses became more serious. Indeed, the transfer became an intolerable burden, and he relinquished his charge of affairs at Woods Hole rather than curtail his own research, an eminently characteristic choice.

In 1908 he tendered his resignation; his letter and the reply thereto follow:

To the Trustees of the Marine Biological Laboratory, Woods Hole,  
Mass.

Gentlemen:

This year has brought the twenty-first birthday of the Marine Biological Laboratory. For these many years you have continued to honor me with the directorship of the Laboratory. In late years I have so far drifted out of office and out of use that a formal resignation at this time can scarcely be more than an announcement of the fact accomplished. The time has arrived, however, when a reorganization seems to be imperatively demanded, and as a prelude thereto, I must ask you to accept this note as a somewhat belated announcement of my resignation of the office of director.

Let me take this opportunity to thank you one and all very heartily for the cordial support you have extended to me.

Respectfully,

C. O. WHITMAN.

August 13, 1908.

The Corporation and Trustees of the Marine Biological Laboratory, in accepting the resignation of the Director, Professor C. O. Whitman, have ordered to be put upon their records and to be forwarded to Doctor Whitman the following minute:

The Corporation and Trustees desire to express to the retiring Director their regret that he finds it necessary to withdraw from the active directorship of the laboratory, and their appreciation of the inestimable value of his services. Since the establishment of the Laboratory at Woods Hole twenty-one years ago, he has been continually its Director and he has to a very large extent guided its growth and development. He has stood for the principles of cooperation and independence which have made the laboratory unique in character and truly national in its reputation and influence. His high ideals and his generous appreciation of the work of others have been an inspiration to the many biologists, who, during these years, have attended the laboratory.

The corporation and trustees desire that the retiring Director may continue to serve the laboratory as honorary director and trustee and that his presence at the laboratory may continue to be an inspiration in the future as in the past.

Professor Whitman's reply was as follows:

To the Corporation and Trustees of the Marine Biological Laboratory,  
Woods Hole, Mass.

Ladies and Gentlemen: Your action of August 13, in which you express a desire to have me serve the laboratory as 'honorary director and trustee' is in itself alone an all-sufficient reward for whatever services I have rendered as Director. Your goodwill is the all-important recompense, and no title that you could confer could add to the weight of your approbation. In fact, titles belittle the spirit. Let me have the latter without the former—without title or office of any kind. Please respect this wish and believe me, as ever, a sincere and devoted friend of the Laboratory.

Respectfully and cordially,

C. O. WHITMAN.

The report of the trustees to the Corporation bearing on Professor Whitman's resignation and on his services to the Laboratory expresses so well what many others feel that it is appropriate to quote it in large part:

Professor Whitman's resignation as Director of the Marine Biological Laboratory, after twenty-one years of service in that position, impressively recalls the inestimable value of his services in the establishment and development of this institution. If we have today one of the lead-

ing marine laboratories of the world, we owe it in large part to him. The interest of almost every member of this board of trustees and of the corporation was enlisted through his efforts, and the splendid influence which the Marine Biological Laboratory has had upon the development of biology in this country is traceable ultimately to him.

His connection with the laboratory began at a time when it had neither permanent home, recognized standing, nor scientific ideals. Some of the leading biologists of this country felt that it could not compete as a research station with the U. S. Fish Commission Station, backed as the latter was by the resources of the government, and that its chief field of usefulness must be as a summer school. Whitman thought otherwise, and by his real greatness as a scientist, his untiring energy and enthusiasm, his splendid ideals and his unfailing faith and courage he made it from the start the principal center in America for biological research.

From start to finish his ideals for the laboratory were these: (1) A national center for research in every department of biology; (2) a laboratory founded upon the cooperation of individuals and institutions; (3) an organization independent in its government and free to follow its natural course of growth and development. For these ideals he has labored consistently and persistently year after year, sometimes with a disregard of present advantage, to be gained by the sacrifice of one or the other of these ideals, which cost him friendships which he highly prized. At one particular crisis he wrote: 'If I have made any enemies through unkindness or injustice, I am sincerely sorry for it; but if I have made any because I have stated my conviction on the question before us I can afford to part with all friends who are made enemies for such a cause.' His faith in the ultimate achievement of these ideals was so great that he chose rather to sacrifice present good than, as he believed, the future welfare of the laboratory; and his plans for the laboratory were so great, while current resources were so small, that he was frequently charged with being impractical. But it is only fair and just to recognize how much was accomplished by adherence to these ideals and to what an extent the spirit and success of the laboratory are due to them.

Woods Hole is indeed a national center for research in several branches, if not in every department, of biology. Whitman had the wisdom to see that biology could progress only as a whole. 'The great charm of a biological station,' he wrote, 'must be the fullness with which it represents the biological system. Its power and efficiency diminish in geometrical ratio with every source of light excluded.' To zoology, which was the only subject represented at first, he added botany and physiology and he strove to make Woods Hole a center in each of these departments. He was one of the first to insist upon adequate provision for experimental work. He was, we believe, the first in this country to plan and plead for a biological farm for the study of problems of heredity and evolution. He desired to make Woods Hole a center for the comparative study of anatomy, pathology and psychology. Some of these lines of work have since been taken up and largely developed elsewhere, but if

Whitman could have had the necessary support in his plans they would have been centered at Woods Hole. This need of a national center of research in every department of biology is still before the laboratory as a living issue, and although this grand concept has so far failed of complete realization, who can say how much the laboratory owes to this catholicity of spirit of its director, how much biology as a whole owes to this splendid ideal?

If the laboratory was to be truly national, Professor Whitman believed that it must be founded upon the cooperation of individuals and institutions; no one man nor institution, however great, could accomplish this purpose. He recognized that common ideals must form the basis of such cooperation, and he sought to bring into close connection with the laboratory every person and every institution that shared these ideals with himself. With these ideals, and by means of his own personal charm and scientific abilities, Whitman secured the cooperation of many of the younger biologists of the country. There was thus developed at Woods Hole a center for research work in biology which has had few equals in the history of the world. By his own work, as well as by his appreciation of the really fundamental problems of biology, he has set a very high standard for the scientific work of the laboratory, and by his kindness, sincerity, and generosity he has called forth similar qualities in others, so that it has been characteristic of Woods Hole, as of few other laboratories at home or abroad, that a spirit of genuine cooperation and mutual helpfulness prevails. Who that experienced it can ever forget the inspiration and enthusiasm of those early years of the laboratory? Who of us can forget the cordial appreciation and generous encouragement which we received from Professor Whitman? Some of us feel that we there incurred a debt of gratitude to him which we can never fully repay. Since those early years other laboratories have arisen and other duties have drawn men away from Woods Hole, but the Marine Biological Laboratory never loses its charm for those who have worked there, and this charm will continue as long as the spirit of cooperation, which Whitman instilled into it, prevails.

Finally, Professor Whitman stood for the complete autonomy of the laboratory. Although aid might have been had more than once from universities and institutions by surrendering the independence of the laboratory, he steadfastly and consistently refused to do this, even though in doing so he had to face the opposition of almost all the members of the board of trustees and the corporation. There is still a difference of opinion as to the expediency of this stand, but there is probably no question as to the desirability of the autonomy. If the laboratory can obtain endowments such as to provide for its present and future needs and to insure its independence we shall all greatly rejoice, but whether it shall succeed in this aim or not, we are probably all agreed that this much at least of Professor Whitman's ideal must be maintained, viz: that the laboratory must be left free to grow and develop as its own needs and the interests of science demand.

These are the ideals which Professor Whitman succeeded in making part and parcel of the Marine Biological Laboratory and which we count among our most valuable possessions. To those who measure the success of an institution by the size of its buildings or endowments, his efforts at Woods Hole may seem in large part to have failed, but those who realize that ideals are the motive forces of the world, that life consists not in abundance of possessions but in abundance of service, that science is not paraphernalia but knowledge—these will not fail to recognize the great value of the work Professor Whitman has done for the Marine Biological Laboratory and for the whole science of biology.

To these words of appreciation there is but little to add. It may be that the Marine Biological Laboratory is Whitman's most enduring monument, as it was his chief work of organization. But the principles will endure eternally, whatever the life of the particular expression they have been given in the Laboratory, and the fact that Whitman was the chief champion of these ideals and that he gave them visible and effective expression is one of his chief claims to affectionate and reverent remembrance.

#### THE AMERICAN MORPHOLOGICAL SOCIETY

Professor Whitman was the leader in the three most important organizations for the advance of zoology in America during the time of his active life: in 1887 he founded the *Journal of Morphology*, in 1888 he became director of the Marine Biological Laboratory, and in 1890 he took the leading part in the foundation of the American Morphological Society. A circular was sent out October 16, 1890, calling on those interested to unite in the formation of an Association of Morphologists "in connection and affiliation with the American Society of Naturalists," which shall hold stated meetings during the Christmas vacation, at which special and general morphological problems may be brought forward and discussed. Attention was directed in the circular to the scientific isolation of zoologists in America, and the advantages of their cooperation in such a society. The committee signing this call consisted of C. O. Whitman (chairman), Henry F. Osborn, E. B. Wilson, E. G. Gardiner, and J. Playfair McMurrich. The first meeting was held in Boston, December 29, 1890. Dr. E. B. Wilson was elected chairman for the meet-

ing. Whitman was then elected president for the next meeting, and was re-elected during the following three meetings. In 1902 the name of the society was changed to "The American Society of Zoologists" and it is still our dominant zoological society.

#### AT THE UNIVERSITY OF CHICAGO

We have departed from the chronological order of events in thus sketching Whitman's various activities. In 1892 Whitman moved from Clark University to the University of Chicago, taking with him the major part of his department and all his students. Professors Mall, Donaldson and Baur also came at the same time from Clark University to the University of Chicago and took part with others in the formation of a department of biology of which Whitman was head. After the first year, however, the department was broken up into separate departments of zoology, anatomy, neurology, physiology and palaeontology. Concerning this event Professor Mall says (The Resignation of Professor Whitman as Director of the Marine Biological Laboratory at Woods Hole, Mass., *Anat. Record*, vol. 2, no. 8, November, 1908).

When the University of Chicago was founded in 1893, Professor Whitman was made head of the biological department, which in its organization was unusually strong on the anatomical side. It was planned at the beginning to divide the department as soon as circumstances would warrant, and with the very rapid growth of the University this took place within a year. Then the anatomical department was established coordinate with those of zoology and botany. This proved to be the most important step in the organization of anatomical departments in America, and for it we are largely indebted to Professor Whitman.

Whitman was in fact mainly responsible for the unusually comprehensive organization of the biological departments in the University of Chicago, and for their establishment in a single group of buildings, thus rendering possible a degree of mutual support and cooperation among the biological sciences, the full possibilities of which have not been realized even in Chicago up to this day. Whitman thus carried out in Chicago as far as possible the same form of organization that he planned for Woods

Hole, involving the representation of every branch of biological knowledge, so as to bring the combined forces to bear on the fundamental problems of biology. It was possible in Chicago to proceed along such ideal lines, for the institution was unhampered by history or tradition or by fixed location of departments established in remote neighborhoods.

The department of zoology under Whitman was primarily a research department and the members of his staff were selected primarily for their standing as investigators. Whitman, himself, taught graduate students exclusively, for the most part candidates for the doctor's degree. He lectured but once a week and not always regularly; but each lecture was a finished essay, and in a way, a piece of original work. He never attempted to present what students could find in books. He consulted about once a week with each student on his research problem, and was a very rigorous and strict critic, but he tended more and more as time went on to let each student work out his own salvation. It often became necessary for the student to seek him at his house for consultation about his work, but such a consultation was always well worth while, as Whitman would leave his own work and play the part of host most delightfully, as well as that of teacher.

The details of departmental administration were very irksome to Whitman, but on the fundamental principles of administration of the department he was firm as a rock and quite uncompromising. Even trifling details would often seem to him contrary to correct ideals, and then the matters of administration loomed large and were rigorously decided.

Whitman's students who took the degree of Doctor of Philosophy under his instruction, with their present academic standing, were the following:

#### 1. At Clark University:

HERMON CAREY BUMPUS, Business Manager, University of Wisconsin.

WILLIAM MORTON WHEELER, Professor of Entomology, Bussey Institution, Harvard University.

EDWIN OAKES JORDAN, Professor of Bacteriology, University of Chicago.

## 2. At the University of Chicago:

HERBERT PARLIN JOHNSON, Associate Professor of Bacteriology, Medical Department of St. Louis University.

FRANK RATTRAY LILLIE, Chairman of the Department of Zoology, and Professor of Embryology, University of Chicago; Director, Marine Biological Laboratory.

ALBERT CHAUNCEY EYCLESYMER, Professor of Anatomy, Director of Anatomical Department, St. Louis University.

WILLIAM ALBERT LOCY, Professor of Zoology, Northwestern University.

HOWARD STEDMAN BRODE, Professor of Biology, Whitman College, Walla Walla, Washington.

CORNELIA MARIA CLAPP, Professor of Zoology, Mount Holyoke College.

AGNES MARY CLAYPOLE (Mrs. Robert O. Moody).

ALBERT DAVIS MEAD, Professor of Comparative Anatomy, Brown University, Providence, R. I.

CHARLES LAWRENCE BRISTOL, Professor of Zoology, New York University.

SAMUEL J. HOLMES, Assistant Professor of Zoology, University of Wisconsin.

JOHN P. MUNSON, Normal School, Ellensburg, Wash.

EMILY RAY GREGORY, Professor of Biology, College for Women, Constantinople, Turkey.

AARON LOUIS TREADWELL, Professor of Biology, Vassar College.

MICHAEL FREDERICK GUYER, Professor of Zoology, University of Wisconsin.

ELLIOT ROWLAND DOWNING, School of Education, University of Chicago.

WILHELMINA ENTEMANN KEY, Lombard College, Galesburg, Ill.

RALPH STAYNER LILLIE, Instructor in Comparative Physiology, University of Pennsylvania.

VIRGIL EVERETT McCASKILL, President State Normal School, Stevens Point, Wisconsin.

JOHN McCLELLAN PRATHER, Teacher of Zoology, Central High School, St. Louis, Mo.

EUGENE HOWARD HARPER, Instructor in Zoology, Northwestern University.

BENNETT MILLS ALLEN, Assistant Professor of Anatomy, University of Wisconsin.

WILLIAM J. MOENKHAUS, Professor of Physiology, University of Indiana.

CHARLES DWIGHT MARSH, United States Department of Agriculture.

JOHN WILLIAM SCOTT, Instructor in Zoology, Kansas State Agricultural College, Manhattan, Kansas.

CHARLES ZELNY, Associate Professor of Zoology, University of Illinois.

LYNDS JONES, Professor of Ecology, Oberlin College.

HORATIO HACKETT NEWMAN, Associate Professor of Zoology, University of Chicago.

JAMES FRANCIS ABBOTT, Professor of Zoology, Washington University, St. Louis, Mo.

VICTOR ERNEST SHELFORD, Instructor in Zoology, University of Chicago.

OSCAR RIDDLE, the University of Chicago.

CHARLES HENRY TURNER, Sumner High School, St. Louis, Mo.

FRANK EUGENE LUTZ, Curator, American Museum of Natural History, New York City.

GEORGE WASHINGTON TANNREUTHER, Assistant in Zoology, University of Missouri.

WALLACE CRAIG, Professor of Philosophy, University of Maine.

CHARLES CHRISTOPHER ADAMS, Instructor in Ecology, University of Illinois.

JAMES THOMAS PATTERSON, Adjunct Professor of Zoology, University of Texas.

MARY BLOUNT, Teacher, University High School, and Assistant in the Department of Zoology, University of Chicago.

KATASHI TAKAHASHI, Professor of Zoology, Gakushinin College, Tokyo, Japan.

MARIAN LYDIA SHOREY, Instructor in Zoology, Milwaukee-Downer College, Milwaukee, Wisconsin.

H. L. WIEMAN, Assistant Professor of Zoology, University of Cincinnati.

GEORGE WILLIAM BARTELMEZ, Associate in Anatomy, University of Chicago.

### WHITMAN'S SCIENTIFIC WORK<sup>6</sup>

Professor Whitman's scientific work covered an unusually broad field. He had a distinct predilection for monographic treatment, and even while engaged on special problems concerning a particular animal or group of animals little escaped his observation or his note-book. Thus his work on leeches was in the first place embryological, but he soon turned to anatomy and taxonomy, and to problems of behavior, all of which are treated in his various publications. Later, in his work on the development of *Amia* and *Necturus*, but little of which has been published, he also paid particular attention to problems of anatomy and behavior. And again, his work on pigeons concerned not only problems of heredity and evolution, but he made an exhaustive study of the taxonomy of the group, and the problems of their behavior were constantly in his mind. He also assigned students problems on their development, and a number of papers were published by Guyer, Harper, Blount, Patterson, and Bartelmez in this field. Another illustration was his plan for a monographic study of *Arenicola* by coöperation of a group of his students, parts of which have been published.

His own publications fall mostly in the subjects of embryology, comparative anatomy and taxonomy, animal behavior, and evo-

<sup>6</sup> This section has been prepared by E. G. Conklin, Albert P. Mathews, T. H. Morgan, J. Percy Moore, and Oscar Riddle. The writer of the main body of the biography has merely prepared the introductory paragraphs.

lution and heredity, an extraordinary breadth of field for a modern zoologist. But whatever subject he touched he illuminated. He was slow to publish, not because of lack of industry or results, but because he was determined to examine the subject to the bottom, and to be sure of his view-point. He rarely had occasion to correct any published statement, and even less rarely, perhaps, to change in any radical way a point of view to which he had once committed himself. Work of such classical distinction could not be very abundant. He has left a large amount of unpublished material, especially bearing on pigeons, though there is much that dates from an earlier period. The statements that follow concerning unpublished material are on Dr. Riddle's authority.

### *Embryology*

In his first scientific publication, "The Embryology of *Clepsine*," Professor Whitman at once took rank as a great zoologist. Although this paper was his doctor's dissertation, it was unusually mature, and showed in striking manner the qualities which characterized all of his later work, viz., patience and accuracy in observation, great power of logical analysis, and a firm hold on large problems. Indeed so fundamental and comprehensive was this work that almost all of Professor Whitman's later embryological work is foreshadowed in it, while it furnished the stimulus for a large amount of work on the organization of the egg and its cell lineage, which was done later by his associates and students at Woods Hole.

Even at this early date (1878) his conclusions as to the organization of the egg and the formation of the embryo were fundamentally the same as his later views. He observed the bilateral symmetry of the egg of *Clepsine* before cleavage; he studied carefully the cleavage of the egg and observed the formation and subsequent history of the ectoblasts, mesoblasts, neuroblasts, and entoblasts; he described the growth and concrescence of the germ bands of *Clepsine*, and compared them with the growth and concrescence of the germ ring of fishes. His conception of the fundamental problem of all development is expressed in these words: "In the fecundated egg slumbers potentially the future

embryo. While we cannot say that the embryo is predelineated we can say that it is predetermined"—words almost precisely like those used by him twenty years later when dealing with this subject.

In 1887 he published another paper on this general subject, entitled "A Contribution to the History of the Germ Layers of Clepsine" in which he extended his former observations on the cleavage, orientation of cleavage planes, origin of teloblasts and germ bands, origin of the mesenteron, and the origin of the ectoderm and its products. And in the same year in a paper on "Ookinensis," he came back to the phenomena of maturation and fecundation, which he had treated in his first paper, and gave a very suggestive and comprehensive review and analysis of these phenomena.

Whitman's general point of view regarding the problems of development are made particularly plain in his biological lectures and addresses. In his address before the Zoological Congress of the Worlds Columbian Exposition, on "The Inadequacy of the Cell Theory of Development" he discussed these problems in a striking and suggestive manner. At this time the work of Wilson and others had just shown the possibility and importance of tracing individual blastomeres throughout the development from the time of their appearance until they give rise to particular portions of the embryo, the cleavage thus constituting "a visible mosaic;" shortly before, Roux had shown that the cleavage of the frog's egg was a "mosaic work;" at the same time the work of Driesch and other experimentalists was leading to a directly opposite opinion. In this conflict of opinion Whitman took a strong and independent position, basing his conclusions not merely on comparative embryology but also upon the comparison of protozoa and metazoa. He protested against the view that organization is the product of cell formation, and insisted that "organization precedes cell formation and regulates it." He contrasted the Cell-doctrine with what might be called the Organism-doctrine. He insisted that, "an organism is an organism from the egg onward, quite independent of the number of cells present," that cleavage is not a process by which organization arises, but that organization precedes cleavage. "The test of organization

in an egg does not lie in its mode of cleavage, but in subtile formative processes. The plastic forces mould the germ-mass regardless of the way it is cut up into cells."

At the same time he showed clear insight into the results of the experimental embryologists. In this same address he says, "The formation of a whole from a part . . . no more disproves the existence of a definite organization in the case of the egg than in the case of hydra." And in the preface to the volume of *Biological Lectures* for 1894 he strongly contests the view that "developmental mechanics" has explained or can explain vital phenomena, without reference to the historical development of the organism. As to mechanism and vitalism he says, "There is no warrant for the assertion that life is something different from, and independent of, matter and energy. That is the mistake of vitalism. On the other hand there is no warrant in decomposition for identifying dead mechanism with living mechanism." "The ultimate mystery is beyond the reach of both mechanism and vitalism; let pretensions be dropped and approximation to truth will be closer on both sides."

The influence of Professor Whitman's work on the science of embryology and on the scientific and philosophical problems connected with development was profound. His work was careful, critical, consistent. He reached conclusions only after most painstaking observations and mature deliberation, and when he had once made up his mind he was not easily moved. Others might be "tossed about by every wind of doctrine," but he stood unmoved and unshaken, having confidence in his own observations and reflections and refusing to doubt his conclusions until he himself had seen and felt equally strong evidence against them. As a result of this he was unusually stable and consistent, and while his later work shows that his ideas were constantly enlarging with new evidence, yet there was little in his earlier work which needed correction.

Professor Whitman's work on the early development of the teleost egg, published with Alexander Agassiz (1884 and 1889), is a fine example of careful discriminating embryological investigation. The cleavage especially was studied with great care, and

the history of the marginal cells led to the solution of "one of the cardinal questions in the early development of the teleostean fishes, namely the precise origin of what His and others have called the 'parablast.'" The authors showed that the nuclei of the periblast, as they termed this layer, are derived entirely from the marginal cells of the blastodisc, and the fallacy of "free cell-formation" and of separate origin of the "parablast and archiblast" thus received its quietus. Their hope that a similar result would be reached for other meroblastic vertebrate ova has since been realized.

### *Leeches*

Professor Whitman's interest in the Hirudinea, aroused and fostered during his occupancy of a table in Leuckart's laboratory at Leipzig, continued unabated until his labors were so tragically terminated. That this interest was no narrow one, but traversed much of the depth as well as the length and breadth of biological science, is much less apparent from a list of the titles of his papers, than from a perusal of their contents. Under such unassuming titles as "The Leeches of Japan," or "Description of *Clepsine plana*," we find treated, not merely specific and faunal details but the much larger questions of the formulation of a new system for standardizing specific descriptions, the morphological basis of generic groupings and the origin, evolution, ecology, adaptations, morphology, classification and paths of dissemination of the land leeches. The latter small group of animals particularly appealed to his naturalist's spirit, not as the disgusting pests that they had seemed to most previous describers and tropical travelers, but as the keen-sensed, facile winners in a competitive struggle for life that must have been of the utmost severity for animals that have departed so widely from the habits and environment of their ancestors.

Although Professor Whitman's contributions to the adult morphology of the Hirudinea were not numerous, numbering only three major papers and seven or eight preliminary sketches, polemics and reviews, they have exerted a great and lasting influence. Taken together they form an exhibit of the catholicity of his interests and exemplify the soundness of his scientific ideals.

Whitman was ever keenly alive to the importance of a many sided view of animal life and was equally sympathetic to the work of the field naturalist and student of ecology and habits, of the systematist, morphologist and physiologist in all their multiform specialties, requiring only that their work should be thoroughly and honestly done.

His earlier papers especially indicate a real and vital interest in systematic zoology and the species question. To whatever wide ranging speculations his studies eventually led him, they received their primary impetus through his efforts to find a satisfactory basis for the discrimination and definition of the species of leeches. Indeed, his very first paper after the publication of his Inaugural Dissertation on the "Embryology of Clepsine," was on "A new species of Branchiobdella" (*B. pentadonta*) which he at that time, in common with other zoologists, regarded as a leech.

To such good purpose did he labor that the history of the systematic study of the leeches may fairly be divided into a pre-Whitmanian and a Whitmanian period. Notwithstanding that he often rode rough-shod over many of the most sacred traditions of systematic zoology, and notwithstanding that his actual determinations of species and genera have sometimes proven unfortunate, nevertheless Professor Whitman discovered the criteria by which evolution and specific radiation in the leeches may best be measured and expressed, and he set a standard for specific description that has since been the guide and model to all the best workers in this field. Out of the chaotic condition that Whitman found order has been wrought, largely through the use of the tools that he devised. It cannot be denied that he imported new light and life to the subject, nor that all later students of leeches have felt the vivifying influence of his ideals.

The key to Whitman's successful analysis of the external morphology of leeches is his discovery (first announced in two preliminary papers published in 1884 and subsequently repeatedly reverted to and expanded) of the segmental sense organs to which he later applied Haeckel's term 'sensillae.' These little, whitish, translucent dots, visible on many living leeches, had been noticed occasionally by earlier observers and Ébrard had even suggested

that they might have a respiratory function, but their real nature and significance as sense organs had passed quite unsuspected.

Whitman showed that these sensillae are groups of tactile cells which differ from the scattered epidermal sense cells chiefly in their aggregation into groups arranged symmetrically according to a definite plan on one ring (now designated the neural or sensory annulus) of every somite and that they are serially homologous with the eyes, which differ from them chiefly in the acquisition of visual cells (Glaskörper of Leydig) and pigment cup.

During the two years (1879–1881) which he spent in Japan as Professor of Zoology at the University of Tokio, Professor Whitman collected material for the description of the leeches of that country. Under his direction most beautiful and accurate colored drawings were made of the species of the several families, but unfortunately the first part only, treating of the Hirudinidae or “ten-eyed leeches,” was ever published. In this paper Whitman’s method of analytical description, based primarily upon the metameric arrangement of the sensillae and the somite composition thus made evident, was first fully applied to the family Hirudinidae. In the course of somewhat elaborate descriptions of the Japanese land leech (*Haemadypsa japonica*), the Japanese medicinal leech (*Hirudo nipponica*) and three species of *Leptostoma* (earlier named *Microstoma* and now known as *Whitmania*) the somites are analysed successively and compared as respects their constituent elements. Comparisons are made with several other European, American and Asiatic genera and the previously unknown or only vaguely apprehended fact brought to light that the metameres of the different genera of ten-eyed leeches are very differently developed as regards the number and size of constituent rings, particularly toward the extremities of the body. These observed differences in the number of rings constituting the somites are correlated with differences in the mode of life of the animals. In addition to their morphological value, these descriptions and the accompanying illustrations are models of beauty and accuracy, than which no more satisfying have been published before or since.

Much attention is given to the description and illustration of the complex color patterns and, although Whitman perceived that in each species all patterns are variants of a single fundamental one, the structural basis underlying them in the arrangement of the muscles and other organs was left to be discovered by his student Arnold Graf, whose tragic end at Woods Hole, Professor Whitman so keenly felt.

The discovery of the neurology of eyes and sensillae was fully elaborated and the structure of these organs minutely described. They were compared to the lateral sense organs of the Capitellidae and the lateral line organs of fishes. The precise determination of the limits and composition of the somites that his method required, led Professor Whitman to the formulation of definite views regarding the nature and history of the metameres, namely:

1. That the neural or sensory annulus is the first of the somite.

2. That each ganglion of the central nervous system supplies the first three rings of one somite and the last two rings of the immediately preceding somites or equivalent portions of somites having less than five rings, and consequently that there is a lack of correlation between definite neuromerism and definitive metamerism.

3. That the quinque-annulate or typical complete somite of the middle region is primitive, and that there is a progressive reduction or abbreviation of the somites toward the ends of the body that is correlated with specialization in other respects.

It must not be understood that Whitman neglected the internal anatomy. On the contrary he was assiduous in the dissection and description of the several organ systems, but except in the case of the nervous system, he added comparatively little to our knowledge.

In a later largely controversial article 'Some New Facts about the Hirudinea,' written in reply to criticism, Whitman forcibly reasserts his views and brings many new facts to their support. In this paper his strong leaning toward the annelid theory of the origin of vertebrates, to which reference is frequently made in later papers, is indicated. He also extends his former opinion

of the homology of the segmental organs with the lateral line organs to include the ear and even the eye of vertebrates, which he believed to have had their origin in organs similar to the sensillae of leeches.

Three years later ('Description of *Clepsine plana*,') we find Whitman with all his enthusiasm applying the same criteria of metamerism and the same methods of analysis to the external morphology of the Glossiphoniidae. This he designated the type somite and derived from it both abbreviated somites having the number of rings reduced to less than three and supplemented somites with the number multiplied to more than three.

In support of these views he also appealed to the facts of embryology and instanced many cases of ring multiplication, incipient or advanced, in various genera of leeches. Each somite of the leeches' body, to a considerable degree, undergoes an independent individual development, the nature and extent of which is correlated with the physiological demands to which it is subjected. But never did Whitman carry out his view to its logical consequences and see, as others have, that the uniannulate and biannulate somites existing at the ends of the body of nearly all leeches are steps toward the elaboration of the triannulate as the latter is toward the quinque-annulate somite.

The relation of sensillae and eyes became clearer and the proof of their homology was buttressed by many facts. Cases of dual sense organs, composed partly of superficial cells bearing tactile hairs and partly of clear visual cells situated more deeply along the course of the optic nerve, and all gradations from typical sensillae with which one or two visual cells and perhaps a little pigment may be associated, to complete eyes with only a trace of tactile cells were described, the latter leading by easy gradations to the strictly visual organs of *Hirudo*. In some cases every step in the transition was found in the successive somites of a single leech, as in *Clepsine hollensis*.

Strong embryological evidence was brought to bear, especially in a paper in the *Zoologischer Jahrbücher* for 1893, as showing the common origin of both kinds of sense cells from common proliferations of the ectoderm, also that the segmental sense organs are

more primitive than the scattered sense cells (goblet cells, labial sense cells and Bayer's organs) and that they cannot have been derived from aggregations of the latter, as had been maintained by Maier and Apathy.

It is most fitting that Whitman's last important paper relating to the leeches should have appeared in the "Festschrift zum siebenzigsten Geburtstage" Leuckarts who had guided his early interest in the group. This memoir on "The Metamerism of *Clepsine*," is the culmination of Whitman's work on metamerism. More than any previous work it is concerned with the nervous system and as an example of complete morphological analysis has few equals among papers dealing with invertebrates. The elements of the central neuromeres and peripheral nerves are correlated one by one with such external features as annuli, sensillae and eyes throughout the body and especially in the simpler somites at the two extremities. The presence in these terminal segments of every morphological element is determined and accounted for and the conclusion reached that complete homodynamy exists throughout. The earlier determination of twenty-six somites in the body of all leeches anterior to the caudal sucker, and of seven in the sucker was confirmed.

Metamerism is traced to the extreme tip of the anterior end, where, however, there is a cephalic region in which the dorsal halves alone of the somites are represented and in which as a consequence, there is a delayed embryonic development. There is no non-metameric residuum at the anterior end and, although acknowledging that embryology furnishes some evidences of the presence of a rudimentary apical organ and remnants of a pair of head kidneys, Whitman denies that there is any element here other than, or added to, the first metamere. There is no prosoma in the sense of Hatschek of an unpaired, non-metameric and premetameric region opposed to the segmented region beginning with the larval mouth. Whitman also contends that these facts confirm the opinion long held by himself in common with Leuckart and others that metamerism originated in multiplication by fission.

*Animal Behavior*

Two papers were published by Dr. Whitman on Animal Behavior, one as a Woods Hole lecture, the other in the *Monist*. There was also a short letter, commenting on an article of Professor Lankester's on the origins of intelligence, published in the *Chicago Tribune*. Of these, the lecture entitled "Animal Behavior" is the most important. It shows him at his best and is one of the ablest of his papers, which is equivalent to saying that it is one of the most admirable papers on this subject. Its style is clear, interesting, and direct; and it may be taken as a model by every investigator. Nowhere else is reasoning more solid and sound, or comment more illuminative. No other of his papers illustrates better the qualities of his genius: the selection of a fundamental problem; painstaking study; publication only after years of observation and reflection; skill in laying bare the simple basis of an apparently complex group of phenomena; a grasp of the subject in all its bearings; and the use of the comparative or phyletic method of attack.

He considers in this paper the fundamental questions of the origin of instinct and intelligence as illustrated by a study of the behavior of three kinds of animals upon which he worked at different periods of his life: the leech, *Necturus*, and the pig-eon.

He begins with the simplest acts of *Clepsine*; its deceptive quiet when disturbed, a quiet of intense rigidity; and its rolling into a ball after feeding when it detaches itself from its host. He shows that the latter instinct can never have been acquired as a habit which has been stereotyped as an instinct, because *Clepsine* feeds but twice or three times in its life. "If the view here taken be correct," he says, "the instinct of rolling into a ball is not a matter of deliberation at all, but merely the action of an organization more or less nicely adjusted to special conditions and stimuli. Intelligence does not precede and direct, but the indifferent organic foundation with its general activities is primary; the special behavior or instinct is built up by slowly modifying the organic basis."

Similarly, the instinct of capturing food exhibited perfectly by the youngest *Necturus* is innate. The pause before seizing the bait is part of a very old primitive mechanism found in fishes and finally developed into the 'pointing' of a dog. Its object is to fix the aim. The timidity of the young *Necturus* is also innate and not the result of painful experiences.

"We have taken a very important step in our study when we have ascertained that behavior, which at first sight appeared to owe its purposive character to intelligence, cannot possibly be so explained, but must depend largely, at least, upon the mechanism of organization. The origin and meaning of the behavior antedate all individual acquisitions and form part of the problem of the origin and history of the organization itself." "We see at once that behavior does not stand for a simple and primary adaptation of a pre-existing mechanism to a special need. As the necessity for food did not arise for the first time in *Necturus*, the organization adapted to securing it must be traced back to foundations evolved long in advance of the species. The retrospect stretches back to the origin of the vertebrate phylum . . .

. . . The point of special emphasis here is that instincts are evolved, not improvised, and that their genealogy may be as complex and far-reaching as the history of their organic bases." This passage should be read by all physiologists who, of all biologists, are most given to neglecting phylogeny in their explanations.

While instinct thus comes before intelligence and not after it, as many have believed, some intelligence was implied by the inhibition of instinctive acts in *Necturus* by fear. To clinch his argument that instincts are evolved like structures and are not inherited habits, he turns to two instincts cited by Romanes as clear evidence of their origin in habits; the tumbling and pouting of pigeons. An examination shows that the rudiments of these instincts are to be found in all species of pigeons. Again the value of the phyletic method of study is illustrated by the 'brooding' instinct of birds. This he shows to be the evolution of an instinct shown even in fishes, which hover over the nest and drive away

intruders. Even Clepsine sticks more firmly than usual when it is over its eggs.

He sums up this part of the paper with a few general statements: "Instinct and structure are to be studied from the common standpoint of phyletic descent and that not the less because we may seldom, if ever, be able to trace the whole development of an instinct. Instincts are evolved, not involved . . . and the key to their genetic history is to be sought in their more general rather than in their later and incidental uses." "As the genesis of organs takes its departure from the elementary structure of protoplasm, so does the genesis of instincts proceed from the fundamental functions of protoplasm."

Taking up now the origin of intelligence he says, "Since instinct supplied at least the earlier rudiments of brain and nerve, since instinct and mind work with the same mechanisms and in the same channels, and since instinctive action is gradually superseded by intelligent action, we are compelled to regard instinct as the actual germ of mind." "We are apt to contrast the extremes of instinct and intelligence—to emphasize the blindness and inflexibility of the one and the consciousness and freedom of the other. It is like contrasting the extremes of light and dark and forgetting all the transitional degrees of twilight." "Instinct is blind; so is the highest human wisdom blind. The distinction is one of degree. There is no absolute blindness on the one side, and no absolute wisdom on the other. Instinct is a dim sphere of light, but its dimness and outer boundary are certainly variable; intelligence is only the same dimness improved in various degrees."

To show how instinct becomes less fixed and choice appears he cites the behavior of three species of pigeons, the passenger pigeon, the ring dove and the common pigeon. If the egg is removed from under the wild pigeon and placed on the side of the nest, the bird returning to the nest remains a moment or two only and then leaves the nest not to return. The ring dove, on the contrary, after some time of perplexity, will return one egg into the nest leaving the other out; the common pigeon, after a longer period of perplexity and uneasiness, will put both eggs back into the nest. There is here a gradual loss of precision of the instinct to leave the

disturbed nest when the rigor of natural selection is relaxed. The common dove is not so much an automaton. Choice begins to appear. "With choice no new factor enters, but only plasticity, so that the pigeon becomes capable of higher action and is encouraged and even constrained by circumstances to learn to use its privilege of choice." "This little freedom is the dawning grace of a new dispensation in which education by experience comes in as an amelioration of the law of elimination. This slight amenability to natural educational influences cannot of course work any great miracles of transformation in a pigeon's brain, but it shows the way to the open door of a freer commerce with the external world, through which a brain with richer instinctive endowments might rise to higher achievement."

"Superiority in instinct endowments and concurring advantages of environment would tend to liberate the possessors from the severities of natural selection; and thus nature, like domestication, would furnish conditions inviting to greater freedom of action, and with the same result, namely, that the instincts would become more plastic and tractable. Plasticity of instinct is not intelligence, but it is the open door through which the greater educator, experience, comes in and works every wonder of intelligence."

### *Evolution*

No account of Whitman's work would be complete without reference to his essay on "Evolution and Epigenesis," not only because this essay reveals him in one of his most thoughtful moods, but because the essay defines very sharply Whitman's attitude toward one of the profound questions of the time—a question that was then engaging the best thought and work of all serious biologists. His keen critical sense is here shown to advantage, his independence of thought led him to break some of the idols of the day, and his thorough understanding of what had been written and was being written at the time, all conspire to make the essay a permanent contribution to our knowledge. He succeeds as few others have done in holding the fine balance between the two extremes of thought represented by the terms pre-

formation and epigenesis. He defines his position in the following words: "I should perhaps say at the outset that I have no theory of development either to announce or to defend. It is of more importance just now to have well-defined standpoints and clear ideas of guiding principles. The possibility, not to say probability, that the egg is from the beginning of its existence as an individual cell definitely oriented has as yet received but little attention." "The drift of opinion, as it seems to me, is neither back to the standpoint of Harvey and Wolff nor to that of Bonnet and Haller, but towards a new standpoint which seeks to avoid the errors and blend the truth of the old hypotheses." Whitman's standpoint is summed up in the two following quotations: "The indubitable fact on which we now build is—the ready-formed living germ, with an organization cut directly from a pre-existing parental organization of the same kind. The essential thing here is—actual identity of germ organization and stirp organization." And again: "Let this organization stand for not more than our neo-epigenesists freely concede, namely, that original constitution of the germ, which predetermines its type of development—let it stand for nothing more than that and obviously the standpoint rises to an altitude scarcely dreamed of in the philosophy of Harvey and Wolff."

Whitman devoted much time to the study of Bonnet's theory of evolution. If one asks why he should have thought it worth while to give so much attention to a discredited theory of the eighteenth century, the answer is first that he wished clearly to point out the error of those "who imagine that they see in recent theories of development a renaissance of Bonnet's evolution" and, second, that "if our theories of development are carrying us back to the standpoint reached by the evolutionists of the last century it is a matter of more than historical interest." His conclusion is "That the old and the new evolution are based on antithetical conceptions which exclude each other at every point." "The old evolution (preformation) was the greatest error that ever obstructed the progress of our knowledge of development. If our examination has helped to clear the mist that obscured important distinctions we have not labored wholly in vain."

*Dicyemids*

In 1882 Whitman published his paper on the Dicyemids. He confirmed much of the earlier work of van Beneden, but made out a different relation between the nematogene and the rhombogene individuals. He discovered certain facts that led him to conclude that the germagene of van Beneden arose from fertilized eggs while all other individuals arose parthenogenetically. Whitman's conclusion in regard to the relationships of the Dicyemids is the same as that to which Hartman has come in his recent monograph.

*Pigeons*

Those who are familiar with Whitman's work regard his studies and experiments on pigeons as his greatest achievement. He died at the very moment when he believed that he had reached a point where he was prepared to publish the results of this extensive and exhaustive investigation. Only on a few occasions (see list of publication 1904-1907) has he stated in briefest outline a few of the principal conclusions he had reached. In a paper read at the Universal Exposition in St. Louis in 1904; in his address before the International Congress of Zoologists in Boston, 1907; and in the Bulletin of the Wisconsin Natural History Society, 1907, Whitman has expressed himself clearly and forcibly on certain fundamental questions of evolution. His address of 1907 has not been printed but the substance of that address is found in his other writings.

The dominant feature of Professor Whitman's long and still unpublished work on inheritance and evolution lies in its intensive and extensive attack upon the nature of a specific character. In the 90's he wrote: "It is to a comparative and experimental analysis of specific characters that we must look for knowledge of the phenomena of heredity and variation." And again, in 1904, in summarizing the results of many years of study of one such character he wrote as follows:

"In tracing the origin and genesis of a single character we meet the leading questions in the evolution of species. First and foremost the question as to the nature of the initial stages. Did the

character arise as a variation *de novo*, or as a progressive modification of a pre-existing character? If *de novo*, did it spring suddenly forth, with some decisive advantage in the struggle for existence? Or did it appear as one of many minute changes, and by some happy chance get a start that gave it the lead in future development? In other words, did it begin as a discontinuous variation, sport, or mutation? Or did it arise cumulatively, as a continuous development? If it originated by modification of an earlier character, was it at first a sudden, sport-like departure? Or was it a slow and continuous transformation, of a progressive or retrogressive nature?

"Then we come inevitably to the deeper question, which natural selection only partially penetrates—the question how variation, multifarious and undirected, without the aid of design or a designer, can advance to such definite and wonderful achievements as specific characters."

Whitman's devotion to the task of learning a specific character knew no bounds; it heeded neither time, personal sacrifice, nor the difficulties which the ensemble of life processes creates when a particular process with which the biologist would become familiar is examined. But, he was ever ready and eager to attend to each and every perturbation of the system, from whatever extrinsic source, if its analysis might lead directly or indirectly to a better measure of realities in his own main sphere of study. It thus happens that along the pathway which he has blazed into the central problems of evolution are to be found many discoveries in the fields of instinct, animal behavior, fertility, correlative variation, the nature of sex, etc.

Having selected color-pattern in pigeons as supplying a satisfactorily small group of specific characters easily accessible to study, he first set about determining which patterns are the more primitive and which the higher and more recent ones, the facts being determined through a most painstaking search for the convergent testimony of the most various kinds of evidence. Here his uncompromising ideal of an intensive and extensive study of a character, his own exceptional mastery of the broad field of zoology, the eighteen years of unbroken and devoted study that

he gave to this work inevitably led him to results of great importance.

A general survey was made of the color-patterns of nearly six hundred wild species, and of nearly two hundred domestic races of pigeons. Large numbers of genera and species from all parts of the world were brought to the breeding pens of his yard. With indefatigable patience the plumage patterns of the living birds were studied; the sequence of pattern in the plumages from young to old was accurately observed; and thoughtful experiments were devised to bridge the gap between the moults, and thus displace apparent discontinuities with visually realized continuities. The primitive pattern of many diverse orders of birds was also ascertained, and the general primitive basis of color-marking in all birds—the ‘fundamental bars’ were discovered.

The direction of the evolution as it was indicated by all these studies was, moreover, again and again retested by evidence of an entirely different sort. Such characters as voice, behavior, and fertility were separately subjected to similar appropriate vigorous comparative and breeding tests to learn whether the resulting data would parallel each other and that furnished by the extensive study of the color-pattern. Only when, by all these means and others, he had accumulated a vast amount of reliable, consistent and convergent testimony as to where the various genera and species stand in the phylogenetic series, did Professor Whitman permit himself to feel that he was reading aright the history of the specific characters of the pattern. And it is a very real monument to his scientific greatness, that, not until he knew all this of the character with which he was working, and much besides, would he write as much as one line concerning it.

In his yards were hybridized nearly forty wild species of pigeons, most of these crosses being made here for the first time. The results of continued breeding of the simple and complex hybrids from these forty pure wild species, and of several domestic races, furnish a mass of most remarkable data. The conclusions from these data being at the same time checked and supported by the results of other lines of study on the same material.

In consequence Professor Whitman's work presents a great body of searchingly self-critical and reliable conclusions, and these conclusions unquestionably lead far into constructive evolutionary theory. For his material he believed he had demonstrated beyond doubt the reality and regnancy of definitely directed variation, i.e., of orthogenesis, as the method of evolution. He has accumulated and presented the most weighty evidences for continuity as against discontinuity in the phenomena of variation, inheritance and evolution. He has thrown new light on the nature and meaning of 'mutants;' such 'mutants' at any rate as occur among pigeons. He accomplished in 1903, and continuously since then, the remarkable result which in Mendelian terms may be spoken of as the control or determination of the dominance of sex and color.<sup>7</sup>

His work was most bountifully and beautifully illustrated, this feature having occupied many years of the undivided attention of excellent artists. Even in the unfinished parts, however, the outlines of the work are so bold and its details of data are so clear as a result of the polishing process to which he, who was the very spirit of clarity and accuracy, subjected them, that time and care will enable others to arrange most of the results in a form that will still carry conviction to the reader.

Whitman's conception of Orthogenesis, and his attitude toward the mutation theory is stated in the following paragraph:

"Among the rival theories of natural selection two are especially noteworthy. One of these is now generally known as *orthogenesis*. Theodore Eimer was one of the early champions of this theory, basing his arguments primarily upon his researches on the variation of the wall-lizard (1874-81). Eimer boldly announced his later works on 'The Origin of Species' (1888), and the 'Orthogenesis of the Butterflies' (1897), as furnishing *complete proof of definitely directed variation, as the result of the inheritance of acquired characters, and as showing the utter 'impotence of natural selection.'* Eimer's intemperate ferocity toward the views of Darwin and Weismann, coupled with an almost

<sup>7</sup> Unpublished data.

fanatical advocacy of the notion that organic evolution depends upon the inheritance of acquired characters, was enough to prejudice the whole case of orthogenesis. Moreover, the controversial setting given to the idea of definitely directed variation, without the aid of utility and natural selection, made it difficult to escape the conclusion that orthogenesis was only a new form of the old teleology, from the paralyzing domination of which Darwin and Lyell and their followers had rescued science. Thus handicapped the theory of orthogenesis has found little favor outside the circle of Eimer's pupils."<sup>8</sup>

"The second of the two theories alluded to is the mutation theory of Hugo de Vries. The distinguished author of this theory . . . maintains, on the basis of long continued experimental research, that species originate, not by slow gradual variation, as held by Darwin and Wallace, but by sudden *saltations*, or sport-like mutations. According to this theory, two fundamentally distinct phenomena have hitherto been confounded under the term variation. In other words, variation, as used by Darwin and others, covers two classes of phenomena, totally distinct in nature, action, and effect. Variation proper is defined as the ordinary, fluctuating, or individual variation, and this is held to be absolutely impotent to form new species.

"Granting that the position with respect to the mutants obtained from the evening primrose (*Oenothera Lamarckiana*) is unassailable, does it follow that *all* new species have arisen by mutation, and that continuous variation has never had, and never can have, anything to do with the origin of species?

"Plausible as is the argument and impressive as is the array of evidence presented, I can but feel that there are reasons which compel us to suspend judgment for a while on this pivotal point of the mutation theory."

Whitman objected strongly to the implication that a variation tendency must be considered to be teleological because it is not orderless.

<sup>8</sup> Whitman.—The Problem of the Origin of Species, Congress of Arts and Science, Universal Exp. 1904.

"I venture to assert that variation is *sometimes* orderly, and at other times rather disorderly, and that the one is just as free from teleology as the other. In our aversion to the old teleology, so effectually banished from science by Darwin, we should not forget that the world is full of order, the organic no less than the inorganic. Indeed what is the whole development of an organism if not strictly and marvelously orderly? Is not every stage, from the primordial germ onward, and the whole sequence of stages, rigidly orthogenetic? If variations are deviations in the directions of the developmental processes, what wonder is there if in some directions there is less resistance to variation than in others? What wonder if the organism is so balanced as to permit of both unifarious and multifarious variations? If a developmental process may run on throughout life (e.g., the lifelong multiplication of the surface-pores of the lateral-line system in *Amia*), what wonder if we find a whole species gravitating slowly in one or a few directions? And if we find large groups of species all affected by a like variation, moving in the same general direction, are we compelled to regard such 'a definite variation-tendency' as teleological, and hence out of the pale of science? If a *designer* sets limits to variation in order to reach a definite end, the direction of events is teleological; but if organization and the laws of development exclude some lines of variation and favor others, there is certainly nothing supernatural in this, and nothing which is incompatible with natural selection. Natural selection may enter at any stage of orthogenetic variation, preserve and modify in various directions the results over which it may have had no previous control."

The particular evidence in favor of orthogenesis on which Whitman rested his case is found in the origin of the bars on the wings of the wild pigeons and on the wings of many domesticated birds.

"The rock pigeons (*Columba livia*) present two very distinct color-patterns; one of which consists of black checkers uniformly distributed to the feathers of the wing and the back, the other of two black wing-bars on a slate-gray ground. These two patterns may be seen in almost any flock of domestic pigeons.

The inquiry as to the origin of these patterns involves the main problem of the origin of species, for the general principles that account for one character must hold for others, and so for the species as a whole Darwin raised the same question, but did not pursue it beyond the point of trying to determine which pattern was to be considered original and how the derivation of the other was to be understood. Darwin's explanation was so simple and captivating that naturalists generally accepted it as final. It is but fair to state that Darwin's conclusions did not rest on a comparative study of the color-patterns displayed in the many wild species of pigeons. Accepting the view generally held by naturalists, that the rock pigeons must be regarded as the ancestors of domestic races, the question was limited to the point just stated."

Between the checkered and the barred types many intermediate stages may be found in different individuals. But which way is the series to be read, from checkers to bars or from bars to checkers? Whitman finds an answer to this question in the evidence from experiments, from development, and from a comparative study of the Columbidae.

"As an experiment, we may take one or more pairs of pure-bred, typically barred pigeons, and keep them isolated from checkered birds for several years, in order to see if the young ever advance toward the checkered type.

"Another experiment should be tried for the purpose of seeing what can be done by working in just the opposite direction. In this case we take checkered birds, selecting in each generation birds with the fewer and smaller checkers, and rejecting the others, in order to see if the process of reduction can be carried to the condition of three, two, and one bar, and finally, to complete obliteration of both checkers and bars, leaving the wing a *tabula rasa* of uniform gray color.

"If these experiments are continued sufficiently far, it will be found from the second experiment that a gradual reduction of pigment to the extreme conditions named can be comparatively easily effected, and that the direction of reduction will always be the same, from before backward; while, from the first experiment,

it will be seen that it is hopeless to try to advance in the opposite direction, from the bars forward to the checkered condition. No variations will appear in that direction, but such as do appear will take the opposite direction, tending to diminish the width of the bars and to weaken their color. It is in this way that we must account for the existence of some fancy breeds in which the bars have been wholly obliterated. The direction of evolution can never be reversed.

"I have tried both experiments for eight years, and as both tell the same story as to the direction of variation, I am satisfied that further experiments will not essentially modify the results."

After tracing wing bars of diverse kinds to checkers, the origin of the checkers was traced from a still earlier and universal avian character.

"It consists of a single dark spot occupying the centre of the exposed part of each feather. In the course of evolution, this spot has been divided into two lateral spots by the disappearance of pigment along the shaft, beginning at the apex of the feather and advancing gradually inward. The old Turtle-Dove character thus passes by a continuous process of division into the Rock Pigeon pattern, consisting of two checkers on each feather, more or less completely separated. The evidences showing such a gradual transmutation are still to be seen, and in such profusion as to wholly exclude doubt. Hundreds of species have been formed in this simple way, leaving no room for the claim of sudden, nontransitional mutations.

"The *transitional* stages between the Turtle-Dove pattern and the checkered pattern of the Rock pigeons, are exhibited not only as we pass from one species to another, but often as we advance from the juvenal to the adult plumage; and frequently they may be seen in different parts of one and the same individual plumage.

"A still older character than the Turtle-Dove spot is seen in the cross-bars, or *fundamental bars*, that appear to mark all feathers of all species of birds. These bars were first noticed in pigeons in the summer of 1903, and were soon found to be common to all species of pigeons and birds in general. From these fundamental feather-bars or their secondary derivatives, a multitude of specific char-

acters have been evolved by gradual modification. The continuity in the evolution of some of these characters can be experimentally demonstrated. The little Diamond Dove (*Geopelia cuneata*) of Australia, owes its small white spots (two in each feather) to these bars. The transitional stages connecting the spots with the bars are not wholly given in passing from the juvenal to the adult plumage. But if we pluck a few of the juvenal feathers at suitable intervals, their places will be filled by new feathers of different ages, and in this way we may get the stages *intermediate between the bars of the young and the spots of the adult*. Thus we see that *the adult pattern, which normally appears to come in as a striking mutation, by a single jump, is only an end-stage in a continuous process of differentiation*. So it is everywhere. Suppression of stages in ontogeny looks like saltations; but whenever we can get at the *history of the character*, we find the continuity comes to light."

#### PERSONAL CHARACTERISTICS

We have described principally the outward events of a life that was not lacking in incident. Many traits of character shine through these events, but the history of his inner life is far from being comprised in such a sketch, and there is no one competent to write it. With Whitman, more than with most men, one felt that the inner life was the dominant factor, that it was genuine, deep and worth knowing; the outward events were more or less accidental; he would have created similar effects under a totally different set of external circumstances.

In person he was of medium height, of stout build and good color in his maturity, though thin and pale in his last years; his hair was snow white from young manhood, his eyes were direct and piercing, his forehead high, broad and noble; he wore a beard and a heavy mustache that somewhat concealed the rather thick-lipped mouth. His bearing was always erect and dignified; his dress was simple and sufficiently conventional, but he entirely eschewed the ceremonial dress and was only once seen in academic cap and gown, and I believe not at all in evening dress.

Whitman's life was simple and studious; it was passed almost entirely between his house and his laboratory. A large part of his work, since 1891 certainly, was done at home, and from about 1895 when he began the study of pigeons, by far the major part. He gradually collected a large number of species of pigeons from all parts of the world, and in the latter part of his life the collection comprised some 550 individuals representing about thirty species. His house was surrounded by pigeon cotes, and he always had some birds under observation indoors, so that the cooing of doves was for years a dominant sound in his house. He took care of the birds for the most part himself, though he usually had the assistance of one or two maids. He thus actually lived with his birds constantly, and very rarely was absent from them even for a single day. He made observations and kept notes on all aspects of the life and behavior of each species, as well as of such hybrids as he was able to produce. He always had one Japanese artist at work continuously drawing pigeons, and for several years two—Hyashi and Toda. Thus he accumulated an immense amount of material for his *magnum opus*, which, however, he was not permitted to finish. It is hoped that a considerable portion of his work may be available for posthumous publication, owing to the self-sacrificing labor that has been put on it first by Dr. R. M. Strong, and then by Dr. Oscar Riddle, one of his students, who is now devoting his entire time to editing the manuscripts.

For many years Whitman carried his pigeons with him to Woods Hole in June and back again in September, as already related. But the burden became intolerable, especially as he always bore the entire expense of his pigeon work personally. He was finally obliged to relinquish the annual trip to Woods Hole and all the cherished associations of the Marine Biological Laboratory. His work probably flourished better under these conditions, but it is to be feared that his health suffered from too close application to work and from lack of variety in his life.

With all of his close application to his study, he was nevertheless most devoted to his friends; he was always pleased to see them, and would spend hours in conversation with them as though he had no other concern in the world. Although he was no smoker

he would always offer cigars and cigarettes, and would frequently light a cigarette himself,—which burned however mostly in his fingers,—to heighten the spirit of hospitality. He rather frequently invited his students or members of his department and other friends to dinner, and then his usually simple meal was changed to a more elaborate repast.

Dr. A. P. Mathews, one of his close friends, writes thus of him,<sup>9</sup>

It is not, however, of his work as a scientist upon which I wish to dwell, but rather to recall his personality that the memory of it may remain always with us. His white hair; his kindling, eager, but thoughtful eyes; his tender, gentle smile; his reticence of speech; his consideration for others; his generosity and courage; his hospitality and graciousness as a host; these endeared him to us all. We shall never forget his simple, unassuming, modest manner; his encouraging sympathy; his ripe and sane judgment. If when he was alone he lived simply, the absorbed student of science, when with his guests in his home he was the embodied spirit of hospitality.

His great influence as a teacher is due in part to his fine example and noble ideals, and in part to his habit of picking out young men, who showed any love for science, inviting them to his home, drawing them out, encouraging them and giving them his friendship. Many of them he helped financially, and all of those fortunate to work near him owe him a debt of gratitude for his sympathy and inspiration. Probably no teacher in zoology since Louis Agassiz has exerted so great an influence on young men.

He was not a faultless man, but his faults were the outcome of his ardent, ideal, uncompromising disposition. He once said to the writer, about the year 1906, that he felt he had been too uncompromising in his beliefs. But it is questionable whether his life would have been so valuable, had his disposition been more pliable. The mood in which he made this remark was a rare one, and it is to be doubted that even had it been more common he could have overcome his native tendency. This quality of course made him enemies who sometimes did not hesitate to express unfavorable opinions in a more or less open manner. But those, who knew Whitman best, know well that he never sought any small personal advantage, and that any appearance of neglect of small matters was due entirely to his absorption in higher con-

<sup>9</sup> Science, N. S., vol. 33, no. 837, pp. 56-58, January 13, 1911.

siderations. He had the courage of his convictions and rarely, if ever, avoided an issue, turned from an opponent, or shunned a fight.

Although Professor Whitman published relatively few papers, he nevertheless occupied a commanding position in science. Some of the reasons have already been indicated. His "eye was single and his whole body was therefore full of light;" his devotion to scholarship was never open to the slightest shadow of suspicion. He was continuously engaged in his personal research which dealt with the most fundamental problems of biology, and he had accumulated vast stores of data, which we hoped he would live to publish himself. But apparently he could never satisfy himself with reference to the fundamental problems on which his mind was fixed; the grand consummation of his work had not come, and he could not reconcile himself to the publication of more or less fragmentary pieces of work. His published papers, mostly short, are models of condensed thought, written in a fine, polished, characteristic style. No less care was devoted to the form than to the substance, and some of his papers will certainly endure as classics of the biology of his time

It was, however, not only his publications, but also his work with his journal, his laboratory and his students, his constant helpful association with other workers, and the example of his austere and studious life that brought him recognition. He never permitted himself to be distracted by the confusion of modern life, social or academic, nor diverted from his steadfast purpose by clamor for quick results.

#### SICKNESS, DEATH AND BURIAL

For several years before his death Professor Whitman suffered considerably from indigestion, and lost much flesh. He was, however, in better health than usual in the fall of 1910. A sudden cold wave came on about December 1, 1910, and Whitman spent the entire afternoon in his yard putting his birds into their winter quarters. In his zeal for his pigeons he forgot about himself. The next morning he was found in a state of coma, and pneumonia

rapidly developed and brought his life to a sudden and unexpected termination on December 6. He, himself, had looked forward to at least ten more years of active study, for apart from his dyspepsia, which he had learned to control very well, his general health was excellent. He thus died unprepared, with work that he had carried on up to the last moment in an unfinished condition.

Memorial services in his honor were held in Convocation Hall of the University of Chicago on December 8, and the same evening his body was taken to Woods Hole in charge of a committee of four appointed by the University, and his two sons. The interment took place in the lot of the Marine Biological Laboratory in the Episcopal Cemetery at Woods Hole in the presence of a small company of scientific friends and colleagues, who came to Woods Hole for this purpose, and some of his friends of the village. His grave lies almost within sight of the institution which he had loved so well, overlooking the harbor.

At the annual meeting of the Corporation of the Marine Biological Laboratory held in Woods Hole, August 8, 1911, the entire body adjourned and marched to the grave of Professor Whitman, where a memorial address was read and a wreath placed on the grave. Thus those who were unable to attend the interment paid their last respects to the memory of the dead leader.

#### LIST OF PROFESSOR WHITMAN'S PUBLICATIONS

- 1878 The embryology of Clepsine. *Quar. Journ. Micr. Sci.*, vol. 18, pp. 215-315.
- 1878 Ueber die Embryologie von Clepsine. *Zool. Anz.*, Bd. 1, p. 5.
- 1878 Changes preliminary to cleavage in the egg of Clepsine. *Proc. Amer. Assoc. Adv. Sci.*, vol. 26, pp. 263-270.
- 1880 Do flying fishes fly? *Amer. Nat.*, vol. 14, pp. 641-653.
- 1881 Zoology in the University of Tokyo.
- 1882 Japanese aquatic animals living on land. *Amer. Nat.*, vol. 16, pp. 403-405.
- 1882 Methods of microscopical research in the Zoological Station in Naples. *Amer. Nat.*, vol. 16, pp. 697-706; 772-785.
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# THE ORIGIN OF THE SEX-CELLS OF AMIA AND LEPIDOSTEUS

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TWENTY-SEVEN FIGURES

## INTRODUCTION

There has been an increasing amount of attention given in the last few years to a study of the origin and migration of the sex-cells of the vertebrates. The number of forms in which this subject has been studied is being constantly extended. While much conclusive work has been done upon the history of these cells in the elasmobranchs, and an equal amount in tracing them in the teleosts, up to this time, they have never been studied in the ganoids. This work was begun over two years ago, and was reported at the 1908 meeting of the Association of American Anatomists in New York. (Allen, '09.)

The material for the present work is obtainable in great plenty within less than half a mile of the grounds of the University of Wisconsin. Since the breeding habits of these two fishes have been thoroughly treated by other writers, it is not necessary to redescribe them. Telleyesniczky's bichromate-acetic fluid and Zenker's fluid have been used as fixing agents and have proved in every way satisfactory. One secret of obtaining good sections is to secure most thorough infiltration by placing the material in a solution of paraffine in turpentine. While turpentine has a bad reputation, no deleterious effects were noted in the course of the work. Paraffine sections were made without difficulty  $7\mu$  and  $10\mu$  thick, and were stained, for the most part, in haemalum and orange G. Heidenhain's iron haematoxylin stain was

sometimes used for the later stages, but showed no superiority over the haem-alum. In the earlier stages of development it could not be used at all, owing to the deep stain that it gives the yolk material.

With the abundance of material and the amount of time given to the work, it was possible to make a careful study of a large series of stages, much larger than it has been found necessary to use in the preparation of this paper.

It is not necessary to enter into a detailed account of the earlier work upon the origin of the sex-cells, because that has already been done in earlier writings. Since the author's articles on the sex-cells of *Chrysemys* and of *Rana*, some important papers have made their appearance, which, with one exception, bear out in a most gratifying manner the conclusions expressed by the writer in the two papers mentioned above and in somewhat less confident manner in the earlier writings of Wheeler, Woods and Beard.

These papers will be discussed in the light of the facts set forth in this paper in the last part of this article, since they are to be considered in a more or less controversial manner.

#### OBSERVATIONS UPON *LEPIDOSTEUS OSSEUS*

*Lepidosteus*, 4 mm. total length. Cells which appear to be sex-cells lie in the ventral portion of the single layered gut entoderm. They can be but dimly distinguished from the other cells of the entoderm among which they lie. They have a more spherical shape than the other entoderm cells, never being flattened as the neighboring cells frequently are. Another difference lies in the fact that the sex-cells contain more and decidedly larger yolk spherules than do the adjoining entoderm cells. Unfortunately these differences are masked by the large quantities of yolk found in the entoderm at this stage. This is true to so great an extent than one can not be certain at this stage as to the identity of the cells in question. At this period the hind gut has a much greater diameter than it has at later stages. At a point one-quarter the distance from its cranial to its caudal end it has a

dorso-ventral dimension of .24 mm. and a transverse diameter of .20 mm.

*Lepidosteus 6.8 mm. total length.* In a similar part of the hind gut of a specimen 6.8 mm. in length the dorso-ventral dimension of the gut endoderm is .084 mm. while the transverse dimension is .056 mm. The total length of the hind gut in this later stage is 1.70 mm. as compared with .76 in the 4 mm. embryo. It is seen that there has been a very decided diminution in the diameter of the gut, and, furthermore, that this is out of proportion to the increase in length. It is correlated with a thickening of the gut wall, due to the drawing together of the component cells.

In the 4 mm. stage the gut entoderm was composed of a single layer of cells, while in the 6.8 mm. stage under consideration its lateral and ventral portions are made up of two, and in some places three irregularly arranged layers of cells, while the dorsal wall is made up of a single layer as in the 4 mm. stage. Two series chosen from several of this stage may be taken as showing typical differences. Both show an advance over the preceding stages in the greater ease with which the sex-cells may be distinguished. This is due to the contrast between the ordinary entoderm cells in which a considerable amount of the yolk material has been absorbed and the more rounded yolk-filled sex-cells. In neither embryo has the process of sex-cell migration commenced. This is clearly evident in one, while in the other there are a very few scattered yolk-filled cells of rather problematical character in the loose mesenchyme above and at the sides of the gut entoderm. One striking individual difference between these two specimens is found in the fact that while in one the sex-cells have retained their primitive position in the ventral and lateral portions of the gut entoderm, in the other they have migrated up into its dorsal portions. Although it is somewhat difficult to establish with absolute certainty this migration from the ventral to the dorsal portions of the gut entoderm owing to the difficulty of distinguishing the sex-cells in the preceding stages, the individual differences in this regard observed in this stage, together with the fairly reliable observations upon earlier stages seem to point to an actual migration of this character.

*Lepidosteus* 7.3 mm. total length. In the single series of 7.3 mm. embryos the sex-cells in general still occupy the ventral and lateral portions of the gut entoderm, having come to lie in the dorsal wall at only a few points, especially toward the cranial end of the hind gut. A very few sex-cells are found to have migrated into the loose mesenchyme between the gut entoderm and lateral plates of mesoderm, occupying positions in it lateral and dorsal to the latter. These migrant cells are merely the precursors of a general migration which does not become conspicuous until the embryo has reached a length of 8.5 mm.

TABLE 1  
*Number of sex-cells in Lepidosteus*

STAGE	NUMBER					TOTAL
	Entoderm	Mesoderm				
<i>mm.</i>						
6.8	No count	15				807
8.6	"	73				
9.2	"	136				
9.3 A	"	41				
9.3 B	"	311				
9.3 C	"	425				
10.7	133	674				
		Int.	Root	R.	L.	
12.0	125	104	163	180	179	751
14.1	128	222	37	197	153	737
17.0	No count			262	235	
18.0	"			171	173	
24.0	"			147	154	

Int.—Intestine.

Root—Root of mesentery.

R.—Right sex-gland.

L.—Left sex-gland.

*Lepidosteus*, 8.6 mm. total length. Passing over several intermediate stages studied, the conditions found in a specimen 8.6 mm. long may be described. At this stage the lateral plates of mesoderm are just beginning to split and to form the coelomic cavities (fig. 7). The interval between the plates is filled with loose mesenchyme, of which that portion lying between the gut entoderm and the aorta will later be condensed by the apposition of the lateral plates of mesoderm in formation of the mesentery.

It will be seen by comparing the figures drawn to scale that the mesentery is at this stage not only relatively but actually much thicker than it is during later stages. The accompanying table serves to show the number of sex-cells and their distribution in certain stages.

In this specimen 73 sex-cells have already migrated out of the gut entoderm into the surrounding mesoderm tissues. While most of them have migrated upward into the loose mesenchyme and splanchnopleuric mesoderm of the anlage of the mesentery, a few have passed laterally into the portions of the splanchnopleuric mesoderm that enter into the formation of the intestinal wall. While the migration of the sex-cells is seen to be well under way at this stage, the great majority of them still remain in the dorsal portion of the gut entoderm. Very few, indeed, are to be found in the ventral half at this stage. The sex-cells of the gut entoderm are easily distinguishable from the other entoderm cells; the latter have lost very nearly all of their yolk material and have become cylindrical in shape. These features stand out in sharp contrast to the large yolk content and spherical form of the sex-cells.

The migration of sex-cells from the gut entoderm into the mesenchyme dorsal or lateral to it may be clearly seen at a few points, as illustrated in figs. 7 and 8. They retain for the most part their spherical form, but cases like that shown in fig. 7 can be readily found. The shape of this sex-cell clearly indicates the mode of progression. They, undoubtedly, pass through the loose network of mesenchyme by an amoeboid movement, however slow or intermittent it may be.

In this stage sex-cells are found in the hind gut from its cranial end to within .2 mm. of the cloaca, a distance of 2.6 mm.

*Lepidosteus 9.2 mm. long.* The number of sex-cells that have migrated out of the gut entoderm is 136 in this specimen. The number of these is still increasing but solely by migration from the entoderm, since there is no evidence of division of the sex-cells during these stages of sex-cell migration.

In this stage the coelomic cavities have appeared in the dorsal portion of each lateral mesodermal plate and the mesentery is

consequently much more clearly defined. In three specimens 9.3 mm. long the following counts of sex-cells outside of the gut entoderm were made:

$$A = 41 \quad B = 311 \quad C = 425.$$

A and C are extreme cases, indicating that the process of migration is an irregular one in point of time. The mesentery in specimen A is .46 mm. wide.

In specimen C those sex-cells destined to migrate out of the entoderm have for the most part already done so, while in A, an embryo of the same stage, the process is just beginning. The coelome is least developed in A and furthest advanced in C. This would indicate that the extent to which the migration of sex-cells has been carried on is correlated with the degree of development of the mesentery, resulting from the enlargement of the coelomic cavities. While these three specimens belong, no doubt, to slightly different stages of development, they were very carefully matched as to length, and are most certainly of the same age.

*Lepidosteus* 10.7 mm. total length. At this period of development, the mesentery is well formed, being much thinner (.18 mm.) than in the 9.3 mm. stage. This results in its possessing a denser texture (fig. 9). The great majority of the sex-cells are scattered through the mesentery, showing no definite arrangement; but lying for the most part in the mesenchyme enclosed between the somewhat denser splanchnic layers of mesoderm. A few are found in the mesodermal layers of the intestine, while a fairly considerable number have remained in the gut entoderm. At this time such sex-cells as are found in any but the dorsal wall of the intestine, at its junction with the mesentery, are most probably destined to remain in their present positions. A few of the sex-cells have migrated to places immediately dorsal and lateral to the root of the mesentery. The latter may be considered to have reached the sex-gland anlagen, although their position relative to the root of the mesentery will be shifted, as we shall see, in the later stages, probably by a general shifting of the tissues in which they lie.

The number of sex-cells which have migrated from the entoderm is found to be 674. It can be fairly taken as the number destined to undergo migration from the entoderm in this particular individual. Those still remaining in the entoderm number 133.

A few scattered sex-cells are found as far forward as the cranial end of the hind gut. The latter is 3.41 mm. in length. Opposite to the cranial portion of the hind gut the sex-cells are rather sparse, increasing in number as one follows the series caudally. They become most numerous a short distance caudal to a point two-thirds the distance from the cranial to the caudal end of the hind gut. The last one in the mesoderm is found at a distance of .46 mm. from the cloaca, and the last one in the entoderm lies at a point .27 mm. from the cloaca.

*Lepidosteus* 12 mm. total length. At this period migration of the sex-cells has progressed to the point where most of them have reached their final positions. They are still to be seen in the entoderm. This number (125) is quite close to that (133) of the similarly situated sex-cells of the preceding stage. The density of the mesodermal tissues surrounding the gut entoderm makes it seem quite unlikely that any more sex-cells could migrate into them from this source.

The distribution of the sex-cells is as follows:

Gut entoderm.....	125	} 392 outside of sex-gland anlagen.
Mesoderm of intestinal wall and Mesentery.....	104	
Root of mesentery between sex-gland anlagen.....	163	
Right sex-gland.....	180	
Left sex-gland.....	179	} 359 in sex-gland anlagen.
Total.....	751	

The table may be allowed to speak for itself. The sex-gland anlagen grade into one another by an intermediate region at the root of the mesentery. More or less arbitrary limits had to be assumed to distinguish between these three regions. In later stages, illustrated by the 17 mm. stage, fig. 11, we shall see

that the sex-cells undergo lateral migration, either apparent or real, so as eventually to lie at some distance on each side of the median point.

The narrowest portion of the mesentery is at about one-quarter the distance from its origin to its insertion. Its minimum width, as measured here amounts to .028 mm., thus showing a great reduction as compared with the 10.7 mm. stage. This reduction in width is shared by the entire mesentery, certain regions remaining broad only on account of the enclosed blood vessels. No doubt the migration of the sex-cells out of the mesentery is in large part responsible for this, but a considerable share of it must be ascribed to the fact that there has been a tendency for the tissues to become more compact.

The total length of the hind gut at this stage has reached 4.03 mm. Sex-cells are found in the entoderm at its cranial end, and from there extend to within 0.62 mm. of the cloaca. The distribution of sex-cells within the sex-gland anlagen is somewhat more restricted, since they extend from a point 0.31 mm. caudal to the beginning of the hind gut, to a point 1.00 mm. cranial to the cloaca. They are rather sparse at these two extremes.

As in the preceding stages, there is no clear evidence of division of the sex-cells, although one can not be absolutely certain upon this point. While at this time many are free from yolk material, others show but little diminution of it. It is true that the sex-cells are often found arranged in clusters, but there is no evidence to show that these are due to repeated division of a parent cell rather than to a tendency for them to congregate through mutual attraction. What the nature of this attraction might be, we do not know; but it might well be akin to that influence which causes the sex-cells to migrate toward the sex-gland anlagen from their source. Similar clusters of sex-cells were found in early stages in *Chrysemys*.

*Lepidosteus 14.1 mm. total length.* Little radical change is to be seen in this stage. The sex-cells were counted and gave the following results:

Gut entoderm.....	128	} 387 outside of sex-gland anlagen.
Mesoderm of intestinal wall and mesentery.....	222	
Root of mesentery between S. G. anlagen.....	37	
Right sex-gland.....	197	} 350 in sex-gland anlagen.
Left sex-gland.....	153	
<hr/>		
Total.....	737	

There is a strikingly close correspondence between the results of the count in this specimen and those in the preceding one. Attention may be called to the fact that in this specimen a materially greater number of sex-cells is found in the right sex-gland than in the left. At the same time there is a very close correspondence in the total number of sex-cells that have reached the sex-gland anlagen as compared with the total number in the 12.0 mm. stage (359).

*Lepidosteus 17 mm. total length.* In this specimen those sex-cells destined to occupy the sex-glands are seen to have migrated some distance to each side of the root of the mesentery, fig. 11. Their position relative to the root of the mesentery and to the Wolffian duct varies at different points along the sex-gland anlage. In the most cranial portion of the latter they lie just medial to the Wolffian duct. As one follows the sex-glands caudally, the sex-cells are found to lie closer and closer to the mesentery, being situated midway between the latter and the Wolffian duct in the middle region of the sex-gland anlage. The most caudally situated sex-cells lie close to the root of the mesentery.

In this and the succeeding stages the intestine had become so voluminous as to make the counting of the sex-cells in its walls very difficult and inaccurate. It is in fact not easy to distinguish them from the cells of the gut entoderm because of their rather small size and their entire lack of yolk material at this stage.

The total number of sex-cells in the sex-gland anlagen of this specimen is rather high, there being 235 in the left sex-gland and 262 in the right. The total number is 497.

The slightly greater number of sex-cells in the sex-glands of this specimen as compared with that in the previous ones is of little significance. It most certainly does not indicate that there has been any extensive division of them. In a previous work upon *Chrysemys*, (Allen '07), it was shown that there was an extreme amount of individual variation in the number of sex-cells. This variation in *Lepidosteus* is relatively slight compared with that observed in *Chrysemys*. In a specimen slightly older than this stage (18 mm.) there were 171 sex-cells in the right sex-gland, and 173 in the left one, the total number, 344, being not far from the average.

In these two stages, 17 and 18 mm., the sex-cells usually occur singly, although in places they are aggregated into clumps so thick as sometimes to show as many as five or six in a section of one of the sex-glands. Whether the sex-cells occur singly or in clumps, they are surrounded by peritoneal cells which contribute materially to the formation of the ridge-like anlage of the sex-gland.

*Lepidosteus 24 mm. total length.* In a specimen of this length, fig. 12, there is no essential advance in the development of the sex-gland. There were 147 sex-cells in the right sex-gland, and 154 in the left one. The total number, 301, is distinctly below the average.

Comparison with other forms leaves no room for doubt as to the identity of these sex-cells. Since the aim of this paper is merely to trace out their origin, we will not follow them through later stages in their history, but will describe the conditions found in a specimen 110 mm., in length, fig. 13. A complete series of sections through the sex-gland region of this specimen was not made, so it is impossible to give a full account as to the number of sex-cells and general condition of the sex-gland at this time. In running through the series one is struck with the sparseness of the sex-cells. Never are more than two or three to be found in a single section, and often none at all. This would lead one to infer that there has been little or no multiplication of the sex-cells even at this late stage of development.

A glance at table 2 shows that there is a general tendency to a reduction in the average size of the cell body in the later stages. This may be due to the absorption of the contained yolk material. There is no marked change in the size of the nucleus.

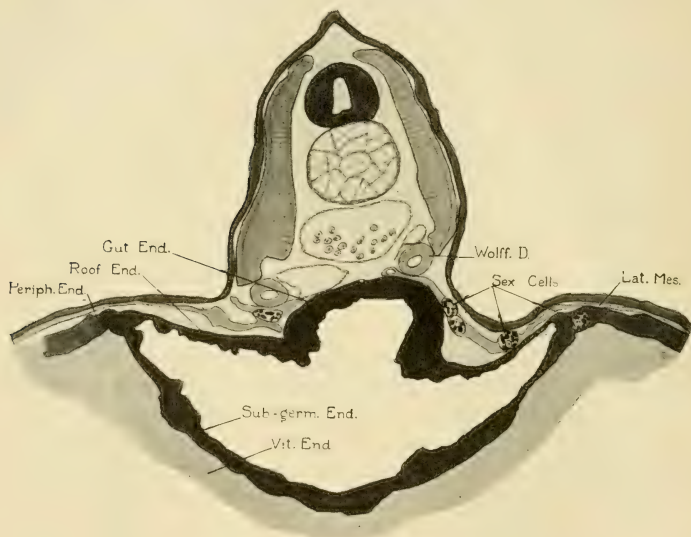
TABLE 2  
*Dimensions of sex-cells of Lepidosteus*

CELL BODY				
Stage	Nucleus	LARGEST	SMALLEST	AVERAGE
<i>mm.</i>				
8.6	6.04	15.10	12.08	13.74
9.3	6.04	18.12	12.08	14.95
10.7	6.04	15.10	11.32	13.59
14.0	5.81	12.08	9.06	10.27
17.0	6.04	13.59	9.06	11.63
24.0	5.81	9.06	7.55	8.65
110.0	6.53	14.50	9.22	12.40

#### AMIA CALVA

*Amia* 4 mm., total length. In the text figure A is shown a transverse section of an *Amia* larva of this stage. It will serve as a starting point from which we shall proceed to consider still earlier stages in tracing out the earliest phases in the origin and migration of the sex-cells. The section shown is taken just anterior to the hind gut, the gut entoderm being clearly marked by its greater thickness and dorsal curvature. The cavity of the intestine at this point opens into the large sub-germinal cavity. The extra embryonic portions of the entoderm, *i.e.*, those which do not form part of the anlagen of the alimentary tract and its appendages can logically be divided into four different regions: (1) The roof of the sub-germinal cavity which is distinguishable from the gut entoderm, as indicated; (2) The layer forming the floor of the sub-germinal cavity; (3) The peripheral layer of entoderm lateral to the sub-germinal cavity (peripheral entoderm); (4) The central yolk mass, or vitellus (vitelline entoderm). In the first three of these regions the cells are arranged in a single layer. They are characterized by the fact that the yolk spherules of the component cells are distinctly smaller than are those of

the vitellus, their diameter being from one-quarter to one-half of that of the typical spherules to the vitellus. In the latter cells are scattered a few of these smaller yolk spherules; but the distinction between the first three divisions of the entoderm and the vitellus is a very sharp one.



TEXT FIGURE A

In connection with this distinction it is interesting to note that the yolk spherules along the cleavage planes that cut through the vitellus are found to belong to this small type. It is easy to see that if the vitellus were cut up into cells as small as those comprising portions 1, 2, and 3 of the entoderm, the thickness of the layers of small spherules which form merely a border to the large cells would be so great as to comprise the entire body of the more finely divided ones. This difference in the size of the

yolk spherules is then probably associated with the difference in the size of the cells. The peripheral entodermal layer which we have designated as division three is interrupted latero-ventrally by blood vessels lying in the mesoderm.

The lateral plates of mesoderm have long since broken away from the mesoblastic somites. Their inner margins lie at some distance to each side of the median line. While there is the slightest tendency in places for the splanchnic and somatic layers of mesoderm to split apart along the medial margins of the lateral plates, the remainder of the lateral plates show no indication of a splitting, even in the arrangement of the nuclei. It is, however, quite probable that such a plane of cleavage is already laid down. This is shown by the sex-cells (text fig. A) being imbedded in the lateral plates. When the somatopleure and splanchnopleure separate later, these will be found to lie in the coelomic cavity, being for a time merely adherent to the coelomic surface of the medial portion of the somatic mesoderm. One can fairly assume that during the period of migration, represented by fig. 5, the sex-cells push their way between the two layers of mesoderm following the potential cleft that separates them.

Text fig. A is very suggestive, as it shows sex-cells situated at intervals from a point just beyond that at which the roof and floor entoderm join the peripheral entoderm. The path of their migration is thus clearly marked out. In this figure it should be noted that the most laterally situated sex-cell lies in the entoderm, while all of the others are clearly imbedded in the lateral plates of mesoderm as already indicated. In but one or two of the many specimens examined were there any sex-cells found in the roof or gut entoderm. They arise in the peripheral entoderm from which they migrate into the lateral plates of mesoderm and through them to their medial borders, whence, as I shall later show, they pass into the sex-gland anlagen after the formation of the coelomic cavity.

The total number of sex-cells found in the mesoderm of the specimen of this stage was 87. Of these 40 were found on the right side and 47 on the left. Text fig. A will indicate their distribution.

Table 3 serves to show for purposes of comparison the numbers of sex-cells found in different specimens of *Amia*.

TABLE 3  
*Number of sex-cells in Amia calva*

STAGE		SPECIMEN	NUMBER OF SEX-CELLS IN MESODERM		
			R.	L.	Total
<i>Hours.</i>	<i>mm.</i>				
132		A	None	None	0
132		B	None	None	0
132		C	None	None	0
132		D	None	None	0
137		A	7	4	11
137		B	9	7	16
137		C	21	8	29
147		A	15	7	22
147		B	14	17	31
147		C	22	11	33
147		D	48	18	66
147		E	39	28	67
147		F	50	26	76
155	3.0		15	34	49
	3.4		62	41	103
	3.5	A	39	53	92
	3.5	B	59	48	107
	3.7	A	42	30	72
	3.7	B	45	47	92
	4.0		40	47	87
	5.0		23	20	43
	5.6		42	56	98
	6.0		28	34	62
	7.0		38	36	74
	7.6		33	42	75
	9.1		36	40	76
	11.4		28	54	82
	15.0	A	28	49	77
	15.0	B	38	45	83
	16.0	A	19	14	33
	16.0	B	22	17	39
	16.0	C			99
	23.7		47	55	102

This stage is a convenient starting point from which to proceed in the study of earlier stages.

*Amia 3.7 mm. total length.* The conditions are, in the main, quite similar to those found in the 4 mm. stage. In one of the two specimens (B) in which the sex-cells were counted there were 92 sex-cells in the mesoderm and 10 in the entoderm. Although this total number of 102 is greater than the number found in the 4 mm. stage (87), yet, as shown in table 2, no significance is to be attached to this on account of the great individual variation in the number of sex-cells observed, not only in *Amia*, but also shown by the author to be so obvious in the turtle, *Chrysemys*. In *A* of this stage, 72 sex-cells were found, 42 on the right and 30 on the left side.

*Amia 3.5 mm. total length.* Two larvae of this stage were studied. It was rather difficult to measure the specimens accurately, owing to the fact that the caudal portion of the body free from the yolk has a strong ventral bend. It can be straightened out only in later stages. The two specimens of this length were taken from the same nest and both are distinctly younger than the preceding, yet they showed decided differences from one another in the positions occupied by the sex-cells, probably owing to the fact that this, in all likelihood, is the period of their most active migration. In specimen *A* the sex-cells are quite numerous in the portion of the lateral plate of mesoderm, which lies immediately above the border of the subgerminal cavity. They occur in fair numbers in the mesoderm between this region and a point one-half the distance from this point to the median edge of the lateral plate of mesoderm. Only three were found nearer the median line than this. Of these, one had scarcely passed the midway point, one was still some distance from the median edge of the lateral plate, while one had actually reached that point.

In specimen *B* of this stage a large proportion of the sex-cells have reached the median edge of the lateral plate of mesoderm of each side. This is especially noticeable on the right side. The conditions in this specimen approach those described for the 4 mm. stage but do not show quite such an advanced condition, owing to the fact that a larger proportion of sex-cells are scattered along the outer portions of what we may call the sex-cell path. There

were noted two or three instances in which the sex-cells were migrating from the peripheral entoderm into the mesoderm.

*Amia*, 3 mm., total length; 155 hours. In a specimen of 3.0 mm. total length, the free caudal portion has but recently separated from the vitelline mass, and has attained a total length of .56 mm. By comparison with a number of embryos of 132, 137, and 147 hours old, the age of this embryo was estimated to be very close to 155 hours. This estimate was made by counting the number of sections passing through the posterior part of the embryo free from the yolk mass. Sufficient numbers of embryos were used to give a fairly accurate determination, there being seven specimens of the 147-hour, three of the 137-hour, and two of the 132-hour stages studied.

TABLE 4

The numbers of sex-cells in each were as follows:

	RIGHT SIDE	LEFT SIDE	TOTAL
In A.....	39	53	92
In B.....	59	48	107

There were 49 sex-cells counted in the 3 mm., 155-hour embryo. This, it will be seen, is decidedly below the average and yet the number is greater than that found in the 5 mm. stage and in the much later 16 mm. specimens.

Only two of the sex-cells have migrated a very short distance along the lateral plate of mesoderm, beyond a point overlying the lateral boundary of the subgerminal cavity; the remainder of them all lie lateral to it. It will thus be seen that they show a much earlier phase of migration than that observed in the 3.5 mm. embryo, not only as regards the number that have migrated into the mesoderm, but likewise in the distance through which they have travelled in their journey in that layer toward the sex-gland anlagen.

*Amia*, 147 hr. stage. That there is a great amount of individual variation in the rapidity with which this migration from the peripheral entoderm to the lateral plates of mesoderm is accomplished may be readily seen by referring to the numbers counted

in the mesoderm of seven specimens of the 147 hour stage. These specimens were all taken from the same nest and kept in the same dish, so there can be but very slight difference in their ages, due, if it exists, to the small difference in the time at which the eggs were laid. It will be seen that the total number of sex-cells in the entoderm in these specimens varies from 22 to 76. The latter number is not only greater than that observed in the 3 mm., 155 hour stage, but almost equals that counted in many specimens of older stages after migration has been completed, as, for instance, the 11.4 mm. and 15 mm. stages (see table). In this stage clearly defined sex-cells can be seen in the peripheral entoderm just below the lateral plates of mesoderm, figs. 16 and 17. These cells are distinguishable from the other entoderm cells among which they lie, by the greater size of their contained yolk granules as contrasted with the small size of the yolk granules in the other cells that make up this layer. The difference is further marked by the more rounded form of the sex-cells. Comparison of these sex-cells in the peripheral entoderm shows them to be identical with other more clearly defined sex-cells in the mesoderm. Of this identity there can be no question, and it is equally clear, from a study of later stages, that these cells, having once migrated into the lateral plates of mesoderm, pass unaltered along the latter to come finally to rest in the sex-gland anlagen. There can be no doubt about the origin of the sex-cells from the entoderm. A number of cases were observed in which the sex-cells were actually in process of passing from the peripheral entoderm into the lateral plates of mesoderm.

At this stage, sex-cells have a wide distribution in the peripheral entoderm, being scattered through a region extending from a point opposite to the region where the blood cells originated to the junction of the peripheral, sub-germinal and roof entoderm. In three specimens of the 137 hour stage, conditions are quite similar to the foregoing. In these embryos the number of sex-cells ranged from 11 to 29. It will be seen that the maximum number of sex-cells counted in this stage is greater than the minimum number of the 147 hour stage, although in all three of these 137 hour embryos, the caudal end of the embryo, that part

that has been lifted off the yolk, is decidedly shorter than in any of the 147 hour specimens.

*Amia*, 132 hr. stage. In four specimens of the 132 hour stage, the caudal end of the embryo was just ready to undergo separation from the yolk. Only in one of them had this really commenced, the separated portion having reached a length of but  $20\mu$ . *Not one of these four specimens showed a single sex-cell in the mesoderm.* There can be no question upon this point because they could be very readily detected if present. In the 137 and 147 hour stages those that migrated into the mesoderm stand out most clearly and sharply from the surrounding mesodermal cells. The points of difference between the two kinds of cells are very striking and unmistakable. The sex-cells on the one hand are large, spherical, have sharply defined boundaries, and are filled with large oval yolk grains; while the mesodermal cells are small, flattened, syncytial, and contain a very few minute yolk granules.

It is very much more difficult to trace the earlier history of the sex-cells in the peripheral entoderm, owing to the slight differences that may be taken as criteria in distinguishing them from the neighboring entoderm cells. Numbers of cells with all the characteristics of sex-cells are found just beneath the anlagen of the blood masses. This stage is just before the development of blood vessels within the embryo, and the blood-forming cells occur in the form of two sharply limited bands, one on each side of the embryo and at some distance lateral to it. Here and there, sex-cells are found in the peripheral entoderm, medial to these areas; but clearly defined cases of this sort are rather rare as compared with the large number seen in this region a little later in the 147 hour stage. It is quite likely that many of these sex-cells are overlooked at this stage owing to the fact that the neighboring entodermal cells contain rather large yolk grains at this time, while those seen in these cells in the 147 hour stage are much smaller than at this stage.

It is quite possible that the sex-cells may migrate medially in the entoderm from an entodermal source beneath the blood anlagen to various points between this region and the edge of the sub-germinal cavity. It is possible that a large proportion

of them may have developed in the peripheral entoderm throughout this entire extent. On the other hand, it is also possible that sex-cells may migrate up into this region from the central entoderm beneath.

We have traced the history of the sex-cells from the 4 mm. stage where they are readily identified by any one who has had any experience in observing these cells, back to the earliest stage at which they are distinguishable in the entoderm. We shall now follow them up to the period when they are enclosed in the definitely formed sex-glands and finally to the stage at which they are found to have begun to increase in number.

*Amia 5 mm., total length.* Passing from the 4 mm. stage to the next represented in our series, 5 mm., we find that the sex-cells have made but little progress in their migration toward the median edge of the lateral mesodermal plates. The total number of sex-cells counted in this stage was surprisingly small, being 43 as compared with 87 in the 4 mm. stage. This difference in number is probably due to individual variation. The hind gut has materially lengthened, being 1.3 mm. in length, compared with .88 mm. in the 4 mm. stage. There has been a corresponding increase in the length of the region over which the sex-cells are distributed. In the 4 mm. stage they extend from a point 0.06 mm., in front of the beginning of the hind gut, caudally to a distance of 0.35 mm. In the 5 mm. stage that we are considering, this region begins at the same point relative to the hind-gut and extends caudally for 0.50 mm., one isolated sex-cell being found at a distance of 0.57 mm. behind the cranial limit of their distribution.

In the more caudal portion of this region the splanchnic and somatic layers of mesoderm have begun to separate to form the coelome. This separation does not at first lead to the formation of a continuous cavity but rather to a series of isolated, somewhat rounded cavities. Further caudad, the coelome becomes more and more completely developed, appearing as a large cavity on each side.

*Amia 6 mm., total length.* At this time the first sex-cells appear in the splanchnopleure just at the entrance of the hind gut.

The first sex-cells in the somatopleure are found in the sex-gland anlagen a short distance (0.04 mm.) behind this point. The sex-cells are distributed somewhat irregularly from the cranial end of the hind-gut to a point 0.90 mm., caudad to this point and there are a few scattering sex-cells still further caudad than this.

The coelome is apparent as a continuous cleft on either side of the hind-gut along the entire extent of the region occupied by the sex-cells. The majority of the sex-cells are to be found in the dorso-medial extremity of the coelome, *i.e.*, near the root of the mesentery. A few lie lateral and ventral to the intestine. The coelomic cleft has not as yet become wider than the diameter of the average sex-cell and we consequently see them usually bridging across it, fig. 18. In no case have they penetrated into the somatic mesoderm as we find them doing later. One sex-cell was found in the gut-entoderm, whither it may have migrated from the mesoderm. It is, on the other hand, quite possible for it to have migrated in the entoderm in the manner of sex-cell migration in the turtle. This is a point of minor significance and an occurrence which is at best very infrequent.

*Amia*, 7 mm., total length. Up to this time, the mesentery has been only potentially present, the two lateral plates of mesoderm being in contact above the gut-entoderm. Now, however, we find that it has begun to elongate and become thin. This is naturally correlated with the increase in the extent of the coelome, fig. 19. Two well defined sex-cells are found in the gut-entoderm, 0.06 mm., cranial to the opening of the hind-gut. These are to be interpreted in the same way as the cell in the entoderm mentioned above. The first sex-cell occurring in the mesoderm is found 0.08 mm. caudad to the beginning of the hind-gut. The sex-cells are distributed through a region extending from a point immediately back of the opening of the hind-gut to a point 1.05 mm. behind it, with a few scattering ones behind these. The total number of sex-cells is 74.

*Amia*, 9.1 stage. Sex-cells first appear .18 mm. cranial to the opening of the hind-gut. They extend from this point to a point 1.59 mm. caudad to this, giving a total extent of 1.67 mm. The total number of sex-cells counted at this stage amounted to

76. Of these all were in the sex-gland anlagen except three; one of which occurred in the gut-entoderm and two in the parietal peritoneum. I am inclined to consider it unlikely for these misplaced sex-cells to reach the sex-glands. One is struck, however, with the great difference in the relative number of misplaced sex-cells in *Amia* as compared with *Lepidosteus*. This may be apparent rather than real, owing to the possibility that in *Amia* large numbers of them may have failed to migrate from the entoderm into the mesoderm during early stages. Owing to the difficulty of certainly distinguishing sex-cells in the entoderm from ordinary entoderm cells, it was quite impossible to make any count of those left behind in migration. All but a very few, however, that reach the mesoderm succeed, as we have seen, in reaching the sex-gland anlagen. A considerable number of cells seen in the entoderm in later stages contain small yolk spherules and show other points of resemblance to sex-cells. In this stage the mesentery has become quite lengthened and the coelome very large. The sex-cells have penetrated into the root of the mesentery, fig. 20.

The sex-cells, with rare exceptions, still contain large quantities of yolk material. In these exceptional cases a finely granular appearance gives at least the suggestion of small unstained yolk spherules. The yolk appears in the shape of particles varying in size from small granules up to large lemon-shaped pieces quite as large as those with which the cells of the yolk entoderm are so completely filled.

*Amia 11.4 mm., total length.* The sex-cells are fairly numerous over a region 1.85 mm. in length, beginning at a point 0.06 mm. back of the yolk stalk and ending at a point 0.85 mm. cranial to the cloaca. Two isolated sex-cells are found caudad to the point named, one of them occurring very close to the cloaca. Their total number in this embryo is eighty-two. The sex-cells have much the same characteristics as in the previous stage.

This stage is marked by a decided increase in the length of the mesentery and by a decrease in the size of the yolk-sac, which is now but 0.7 mm. in diameter and is greatly hollowed out to form a portion of the intestinal wall.

While the sex-cells of the 9.1 mm. stage are imbedded in the mesoderm at the root of the mesentery and always close to the median line, they are found in the 11.4 mm. stage to occupy a position a short distance on each side of this point. Not only have they moved laterally, but they have also protruded into the body cavity, accompanied by a few mesoderm cells which are intercalated between them, fig. 21, and surround them with a thin peritoneal investment as well.

*Amia 15 mm., total length.* In this stage the sex-cells extend over a distance of 2.70 mm. in the caudad 0.50 mm. of which they are very sparse. The sex-glands protrude further into the body cavity than in the preceding stage, and the ligament of attachment becomes narrower. The genital ridge is very much lower in the gaps between sex-cells than it is in the sex-cell regions. In spite of the fact that it may be very low for quite a distance, it is continuous throughout. The genital ridges diverge quite widely at their cranial ends, approaching the median line at a point .4 mm. caudad to their point of commencement.

The sex-cells have almost uniformly used up their contained yolk material, although a few scattered ones are still closely packed full of them. The sex-cells in specimen A, numbered 28 on the right side and 49 on the left, the total number being 77. The number of sex-cells in specimen B was 38 on the right side and 45 on the left, the total being 83.

*Amia 16 mm. long.* In two 16 mm. larvae, conditions very similar to those of the 15 mm. stage were found. None of the sex-cells contained yolk material in a sufficiently large amount to be clearly recognizable. The striking thing about these two specimens is the very small number of sex-cells present, 33 in one case and 39 in another. There is no indication of degeneration or of a failure to migrate to the proper positions. The case seems to be similar to one cited in *Chrysemys*, both being due to individual variation.

These two specimens were taken from the same brood and no doubt had the same parentage. Another 16 mm. specimen taken from a different brood showed 99 sex-cells, a number not very far below the maximum. From this fact, and from the

total absence of any indication of degeneration of sex-cells in these or earlier stages, I feel convinced that this small number does not indicate any tendency to degeneration of sex-cells.

*Amia*, 23.7 mm. total length. In the next stage studied, 23.7 mm., the sex-cells numbered 102. Here again there is no evidence of a change in the number of sex-cells originally present. The number, although somewhat high, is exceeded by some of the specimens of very much earlier stages. There is no evidence of sex-cell division nor of any degeneration.

*Amia*, 40 mm. total length. At this stage the sex-gland is elongated oval in transverse section. It has become bent over in such a way that the proximal edge is medial and the free edge

TABLE 5  
*Dimensions of sex-cells of Amia*

STAGE	NUCLEUS	CELL BODY
<i>Hours</i>		
137	7.10	18.03
147	6.71	18.70
<i>mm.</i>		
3.7	6.45	21.88
5.0	6.51	17.80
9.1	8.00	14.96
11.4	7.48	11.59
15.0	7.48	12.64
16.0	7.74	14.06
23.0	7.22	14.20

lateral in position. The mesodermal cells have increased greatly in number. The peripheral cells have become arranged into a somewhat poorly defined layer, while the sex-cells lie in the interior of the sex-gland. No attempt was made to determine the time at which the sex-cells begin to divide, or to study the further development of the sex-glands.

Measurements of the nuclei and cell bodies of the sex-cells gave the following averages, two diameters being measured in each of five sex-cells chosen at random in each stage.

Although the number of cells measured in each stage is hardly sufficient to justify one in considering these average dimensions

to have any high degree of accuracy, I feel that we are quite justified in concluding from these figures that: (1) there is a fair decrease in the size of the cell-body as development proceeds, and (2) that there is a slight increase in the size of the nucleus.

The decrease in the size of the cell-body is probably due to the absorption of the yolk material with which the sex-cells are so richly filled during the earlier stages. No good explanation to account for the slight apparent increase in size of the nucleus presents itself.

#### DISCUSSION OF RESULTS

We can not consider this work as completed without making a comparison between the sex-cells and the other cells of the embryo. This subject will first be taken up in *Amia* where we have traced the sex-cells back to earlier stages than in *Lepidosteus*. It has already been pointed out that the sex-cells, as first seen in the peripheral entoderm, are to be distinguished only by the size and arrangement of the yolk spherules. The nuclei bear a close resemblance to those of surrounding cells of the same size, while the larger nuclei of larger cells show many points of similarity to them. In all except the earliest stages studied, these nuclei are quite rounded. The chromatin appears in the form of slender strands that take a peripheral position in the nucleus. There is invariably a plasmosome present and rarely two of them. In the 147 hour stage the nuclei of the sex-cells bear a resemblance not only to those of the neighboring cells but also to those of the gut entoderm. In fact, many nuclei of the mesoderm show similar characteristics.

After development has gone a little further, as in the 3.4 mm. and 4 mm. stages, the mesodermal nuclei and those of the gut entoderm are found to have become smaller and are more deeply stained than those of the sex-cells and peripheral entoderm. In all of these later stages, which include 5 mm., 6 mm., 9.1 mm., 11.4 mm. and 16 mm. larvae, these differences are found to increase. Although the sex-cells undergo a migration from the peripheral entoderm into the lateral plates of mesoderm and through the latter to the sex-gland anlagen, they still bear a close resemblance

to certain cells of the peripheral entoderm. This not only involves a similarity of the nuclei but of the dimensions of the cell bodies. This is true even after the sex-cells and the corresponding cells of the peripheral entoderm have lost their yolk through absorption.

In the stage of 11.4 mm., the yolk mass has been greatly reduced (figs. 25 and 26). Only here and there about its periphery are cells to be found with well defined outlines. The great mass is syncytial, with large nuclei of varying size scattered here and there. While these nuclei of the vitelline mass are much larger than the sex-cell nuclei, they bear a close resemblance to the latter. The nuclei of the well defined peripheral cells are practically identical in size and appearance with those of the sex-cells.

While the similarity between sex-cells and between these two classes of cells is not so marked in *Lepidosteus* as in *Amia*, yet it appears to be equally true. In the 17 mm. stage (figs. 14 and 15) the yolk mass is still of fair size. There is a layer of peripheral entoderm that is largely made up of cells with clear boundaries, whose nuclei are similar to those of the sex-cells in respect to the presence and character of the plasmosome and in the form and distribution of the chromatin material. In many cases these nuclei are larger than those of the sex-cells; but many are found which are quite as small. These grade into the very large nuclei of the syncytial vitelline entoderm.

At this stage the tissues of the body have taken on their distinctive characters and their component cells have undergone in many cases a high degree of specialization. This emphasizes strongly the similarity between the sex-cells and the cells of the peripheral entoderm.

As we pass back to earlier stages, such as those of 9.3 mm., 5.9 mm., etc., we still find this similarity between these types of cells, although the nuclei of all the body cells tend to show greater and greater similarity to one another in the earlier stages. For instance, it becomes quite difficult to distinguish the nuclei of the gut entoderm cells from those of the sex-cells. Even the nuclei of the Wolffian ducts show quite a close resemblance to the sex-cell nuclei during the early stages of development.

There are two ways of viewing the similarity that the sex-cells of *Amia* and *Lepidosteus* bear to these cells of the peripheral entoderm. The well defined cells of the peripheral entoderm might be interpreted as sex-cells that have failed to migrate into the lateral plates of mesoderm. It would then remain to give an explanation of the resemblance that the nuclei of these cells bear to the nuclei of the vitelline entoderm and to account for the intermediate types of nuclei by which they grade into one another.

The other view of this problem is to consider sex-cells, peripheral entoderm cells, and vitelline entoderm cells as slightly differentiated blastomeres, dating from an early stage of development, and to consider the similarity that they bear to the cells of the peripheral entoderm as due to the fact that they too have remained in a relatively slightly differentiated condition. This view seems the more probable of the two. It is by no means a new one, having been advanced by Nussbaum in 1880.

It would be rash in the extreme to claim that the sex-cells might not differ in some essential chromosomal characters from the cells of the peripheral entoderm which they so closely resemble, and yet careful study has failed as yet to show any real differences. While such differences may exist, these cells all have much in common with one another.

In a recent paper by A. P. Dustin ('07), this author gives a new view of the origin and movements of the sex-cells of *Triton alpestris*, *Rana fusca* and *Bufo vulgaris*. Since his view is so greatly at variance with my own, it will be necessary to review this work in some detail. He begins with an account of the sex-cells of *Triton*, and stress is laid upon this form, the author showing a strong tendency to bring his studies upon *Rana* and *Bufo* into line with his work upon *Triton*.

He first recognizes the anlage of the sex-cells in the medial portions of the lateral plates of mesoderm in the 3 mm. larva of *Triton*. They occur only in the caudal half of the body and involve only those parts of the lateral plates of mesoderm lying medial to the Wolffian ducts. In the early stages these cells are filled with large yolk spherules and do not greatly differ from the mesodermal cells that surround them. At a later period the sex-

cell anlagen are pushed together in the median line, between the aorta and the roof of the archenteron. They fuse into a median longitudinal rod of cells lying just above the dorsal root of the mesentery. By this time the sex-cells have lost their yolk material and have, to a large extent, assumed their definitive character. During these stages the number of the sex-cells has increased from one hundred to one hundred and fifty, occasional mitoses being observed. Soon after this stage of the median anlage (9 mm.) has been reached, the sex-cells migrate laterally to their final positions on each side of the root of the mesentery. At the stage of 14 mm., a large number of them degenerate, leaving only 60. A second generation of sex-cells soon begins to form from a source entirely different from the first, namely, from a transformation of ordinary peritoneal cells. Dustin is, in this regard, quite in accord with Bouin who expressed similar views regarding *Rana*. Dustin considers somewhat more briefly the corresponding stages in *Rana* and *Bufo*. Here he finds what he considers to be a substantially similar source of origin of the sex-cells, namely the medial borders of the lateral plates of mesoderm. An incredible feature of his account is the statement that the lateral sex-gland anlagen contain no sex-cell at all comparable in size to those of the yolk-filled entoderm, at the period immediately prior to their union in the median line. Dustin would have us believe, nevertheless, that these selfsame sex-cells show a close resemblance to the entoderm cells immediately after this union of the lateral anlagen, and this in spite of the fact that both of these stages of development are so close together that the embryos upon which he made these observations were all of the same length. His own statement is as follows:

“Au moment où les ébauches paires séparées par une sorte de clivage des lames latérales du mésoblaste se sont rapprochées de la ligne médiane, les cellules sexuelles futures passent par une série de transformations cytologiques à la suite desquelles elles auront presque les caractères des cellules de l’hypoblaste vitellin. Les dimensions des corps cellulaires augmentent dans de fortes proportions; les grains vitellins deviennent beaucoup plus nombreux et plus volumineux; ils se colorent mieux par l’orange G. Par le fait de l’augmentation du nombre des plaquettes

vitellines, le noyau, souvent réfoulé à la périphérie de la cellule, présente à sa surface une série d'encoches lui donnant un aspect hérissé (p. 476).

He finds the number of sex-cells in *Rana* to increase gradually, from 75 in the 8 mm. stage to 90 in the 15 mm. stage, at which time sex-cells begin to be formed by the transformation of ordinary peritoneal cells. Simultaneously there is a degeneration of sex-cells which is overbalanced by this process of transformation.

In criticism of the above views I wish, first of all, to admit the possibility that Dustin may be perfectly correct in his account of the origin of the first line of sex-cells from the lateral plates of mesoderm in *Triton*. His account of this feature is circumstantial and rather convincing. His account of a transformation of peritoneal cells into sex-cells during later stages is by no means so easy of acceptance. His figures to demonstrate this are not convincing.

His counts of sex-cells are not given in any circumstantial detail and there is no indication as to whether the number of sex-cells recorded for any given stage is the result of a count of the sex-cells in one specimen or in several. One can not be blamed for being skeptical of the value of such counts if made upon but one specimen of each stage, when so few stages are chosen to demonstrate general processes of degeneration and new formation. Such a process can only be established by a count of the sex-cells of numerous specimens.

I wish to express my complete disbelief in the first appearance of the sex-cells in the lateral plates of mesoderm of *Rana* and *Bufo* in the manner described by Dustin. In my paper upon "An Important Period in the History of the Sex-Cells of *Rana pipiens*" ('07) I showed that the sex-cells migrate upward from the median dorsal portion of the gut entoderm at the time when the two lateral plates are pushing together to the median line in the process of forming the mesentery. Attention was called to the resemblance that this process bears to an actual pinching off of the mass of sex-cells by the inner margins of the plates of

mesoderm. As pointed out in my article, the lateral plates of mesoderm, examined immediately before their approximation in the median line, show no cells which, as regards size or yolk content, in the least compare with the sex-cells.

It is especially gratifying to me to find support for my views in two recent papers. In one of these Kuschakewitsch ('08), referring to my paper of a few months before, stated: "Der Verfasser hat die Abschnürung von Dotterzellen längs der dorsalen Sagittallinie des Dottersackes im hinteren Teile des Rumpfes beobachtet und die Theilnahme dieser Dotterzellen am Aufbau einer kompakten Mesenterialanlage festgestellt, die Bouin (1900) als "ébauche génitale primordiale" aufgefasst hatte. Wie aus meiner Schilderung der entsprechenden Vorgänge in der Normalreihe von *Rana esculenta* zu ersehen ist, kann ich die Angaben von Allen vollständig bestätigen."

Another paper, appearing the same year (King, '08), gives an account of the origin of the sex-cells in *Bufo lentiginosus* which is in complete accord with the above, and states: "Allen's recent account of the origin of the sex-cells in *Rana pipiens* agrees essentially with what I have found in *Bufo*." Miss King finds no evidence in the course of development of any transformation of peritoneal cells into sex-cells as asserted by several writers among whom may be mentioned Bouin and Dustin. This is quite in accord with my observations upon *Chrysemys* ('06) in which the sex-cells were traced to the period of sexual maturity without finding any evidence of such transformation.

Miss May Jarvis ('08) in a paper upon "The Segregation of the Germ-Cells of *Phrynosoma cornutum*" (preliminary note) finds the sex-cells to take their origin in the entoderm of the vascular area on all sides of the embryo, even cranial to it, and notes a few in the region of the brain. Her results are in their main features confirmatory of my own work upon *Chrysemys*. The following quotation from her paper is self-explanatory: "Through the courtesy of Dr. Allen, I have been enabled to examine the more important stages in the migration of the germ-cells of *Chrysemys*; they are similar to my own material, as my conclu-

sions, although differing from Dr. Allen's in details of early distribution and periods of migration, uphold his."

Rubaschkin ('08 and '09) in a couple of recent papers, has shown that the sex-cells of the rabbit and guinea-pig are first to be found in the entoderm at some distance on each side of the hind-gut and that they follow a path almost identical with that followed by the sex-cells of *Chrysemys*. These references to the coincidence of the views of other recent writers with my own are made to show that I do not stand alone in placing emphasis upon the entodermal origin of the sex-cells in the vertebrates. At the same time I wish, however, to disclaim any intention of making at this time a sweeping claim that the sex-cells of all vertebrates arise in the entoderm. Wheeler's work on *Petromyzon* ('99) shows that they may be included in the mesoderm at the time when that layer is split off from the entoderm. He has, however, pointed out their similarity to the entoderm cells and their dissimilarity to the mesodermal cells among which they lie.

I do not seek to discredit the work of Dustin upon the sex-cells of *Triton*; although his statements about the origin of the sex-cells in *Rana* and *Bufo* strike me as being very far from the mark, because they are so radically at variance with not only my own observations, but with those of King and Kuschakewitsch as well. Dustin, in his attitude toward the work of others, seems to consider that there must be a strict uniformity in all forms in both the place of origin and in the movements of the sex-cells. He has apparently studied this problem first in *Triton* and at some length. His results, probably correct for that form, he has attempted to apply to *Rana* and *Bufo* as well, undeterred by the difficulties to which attention was called above. Dustin is quite ready flippantly to dismiss my work upon *Chrysemys*, because the results there expressed did not coincide with the views that he had formed regarding the origin of the sex-cells in *Triton*, *Rana*, and *Bufo*.<sup>1</sup> The process of migration through the entoderm is so clear in *Chrysemys*, that it is unmistakable. The sex-cells are not only characterized by their larger size,

<sup>1</sup> See postscript.

definite, rounded outlines and fine chromatin network, but by their large yolk content and the fact that they do not divide during the stages in dispute.

The sex-cells are migratory to a high degree. The path and time of their migration may vary greatly within a given group of animals, as illustrated by the case of *Amia* and *Lepidosteus*. While in the forms that I have studied they are first to be observed in the entoderm, I am quite open to conviction that in other forms they may migrate from this layer into the potential mesoderm before the two layers are separated, as shown by Wheeler in *Petromyzon*. It is even conceivable that they may lie, from the very beginning of development, in material destined to form mesoderm—that they may never have existed among cells actually or potentially entodermal. The more recent development of our work along these lines, however, most certainly tends to show that it is usual among the vertebrates for the sex-cells to first appear in the entoderm.

#### SUMMARY AND CONCLUSIONS

1. The sex-cells of both *Amia* and *Lepidosteus* have their origin in the entoderm. In *Amia* they are first distinguishable in the peripheral entoderm from the lateral angle of the subgerminal cavity to the anlage of the blood cells.

In *Lepidosteus* they are first seen in the ventral and lateral portions of the gut-entoderm, although analogy with *Chrysemys* leads us to assume that they may have migrated through the entoderm to these regions from more lateral anlagen, similar to those from which the sex-cells of *Amia* arise. In both forms, the sex-cells arise only in the region of the hind-gut. None were found at any considerable distance in front of it.

2. The path of sex-cell migration in *Amia* carries them out of the peripheral entoderm directly into the overlying lateral plates of mesoderm, along which they travel, to come to rest near the medial edges of the latter. These portions are destined to join above the intestine to form the mesentery. As the splanchnic and somatic layers of the lateral plates of mesoderm

split to form the coelome, the sex-cells adhere to the somatic layer at a point near the root of the developing mesentery—the sex-gland anlage. They later sink into the peritoneum of this region, which afterwards proliferates to form a long ridge—the sex-gland. Very few sex-cells fall by the wayside in this migration, practically all reaching the sex-glands.

3. In *Lepidosteus* the sex-cells, first seen in the ventral and lateral portions of the gut-entoderm, migrate to occupy a position in the dorsal portion of it, from which they pass dorsally into the loose mesenchyme that forms the substance of the developing mesentery. As the mesentery becomes more narrow and compact, owing to the increase in size of the body cavity, the sex-cells migrate to its dorsal portion and laterally to the sex-gland anlagen. Roughly speaking, one-half of the total number of sex-cells reach the sex-gland anlagen, the remainder being distributed between the intestinal entoderm, the mesodermal layers of the intestine, the mesentery and the tissues at and dorsal to the root of the intestine.

4. The number of the sex-cells in *Amia* and *Lepidosteus* is a matter of individual variation for those periods of development during which they do not undergo division. The average number in *Amia*, after the period when the migration from the entoderm to the mesoderm has been completed, up to the latest stage in which counts were made, was found to be 75. In *Lepidosteus* it was 765, an average of 636 of these occurring in the mesoderm.

5. There is a close resemblance between the nuclei of the sex-cells and of the yolk cells. This is especially true of certain cells of the peripheral entoderm, although these grade by gradual transition forms into the large nuclei of the vitelline entoderm. This is probably due to the fact that both types of cells have undergone but little differentiation in the course of development.

#### POSTSCRIPT

A few days before proof of this article came to hand, I received, through the courtesy of the author, a reprint of an article by A. P. Dustin, entitled, “L’Origine et l’Evolution des Gonocytes chez

les Reptiles," (Archives, de Biologie, 1910). This article deals with the origin of the sex-cells in *Chrysemys marginata*, the form which served as a subject for my own work of 1906. As noted above, Dustin in his paper "Recherches sur l'origine des gonocytes chez les Amphibiens" 1907, exhibited scant respect for my work on the sex-cells of *Chrysemys*. It was, no doubt, in large part, this feeling that prompted him to repeat my work. While he, no doubt, expected to find in this form a confirmation of his previously expressed views, he is led to substantiate completely my statements regarding the entodermal origin of the sex-cells. He traces them along the same migration path that I demonstrated four years before. For all this he now gives me full credit and support; but takes issue with my statements regarding the distribution of the sex-cells prior to their migration into the embryo, and, furthermore, claims to have evidence to show that there is a new formation of sex-cells, due to a transformation of ordinary peritoneal cells. These points of controversy and certain other minor ones can not be considered here, but I promise a full discussion of them in another place. I may say that I am fully prepared to maintain my views upon all of the points at issue.

On my part, the work that I have carried on upon *Necturus* since this paper was written, has given me results quite similar to those at which Dustin arrived in his work upon *Triton*. I may say that preliminary studies have convinced me that the sex-cells arise in an essentially similar manner in *Amblystoma*. We then see that, in all three of these urodeles, the sex-cells arise from the inner edges of the lateral plates of mesoderm. I owe it to myself to call attention to the fact that I have at no time disputed the accuracy of Dustin's work upon *Triton*. While the evidence seems to me quite clear that this is the usual, if not the universal, mode of origin of the sex-cells among the urodele amphibians, I am ready to maintain with equal vigor the entodermal origin of the sex-cells in the aruran amphibians, at the same time admitting the possibility that exceptions to this apparent rule may be discovered. I do not feel however, that Dustin has proved his case in *Rana fusca* and *Bufo vul-*

garis. The discussion of his work above gives the reasons for my position in this matter.

Not only does it seem probable that the sex-cells arise during early stages in the mesoderm of the urodeles, but this seems to be the case in the teleosts as well. The most recent and satisfactory support of this view is contained in the excellent paper of Dr. Gideon S. Dodds upon the "Segregation of the Germ-Cells of the Teleost, *Lophius*," in the *Journal of Morphology*, 1910. Here again, we must urge caution in forming a sweeping generalization from the facts thus far at hand. There is certainly a wide field for work in the study of the origin of the sex-cells of the vertebrates. It is a subject which should be approached in a spirit of broad toleration for the views of others. The sex-cells are cells that retain their early embryonic character after the somatic cells have undergone specialization. It seems, from a number of observations made by different authors, that in most forms the sex-cells first make their appearance in the entoderm—the germ layer whose cells appear to maintain their primitive embryonic characters longer than do those of the other germ layers. At the same time, unimpeachable evidence shows that this apparently logical process is not universal, and I have at no time claimed that it is. The sex-cells, as shown by Nussbaum, Eigenmann, Beard and others, do not belong to any one germ layer, but are, in a sense at least, independent of the somatic tissues. They are free to follow their own path in their travels from the place of origin to the sex-gland anlagen, where they finally come to rest. While this path is no doubt identical or similar in closely allied species and in more general divisions of the vertebrates, I do not feel that we are justified in attributing a high degree of phylogenetic importance to the different steps in the migration paths through which they travel.

I wish to express my indebtedness for the work of our departmental artists, Misses Hedge and Battey. I am indebted to Miss Hedge for the execution of diagrams 1-6 and for figs. 9, 10, 14, 15, 21, 22, 25 and 26; and to Miss Battey for figs. 11, 12, 13, 23, and 24. The remaining drawings are my own.

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# ABBREVIATIONS FOR ALL FIGURES

<i>Arch.</i> , Archenteron	<i>Periph. End.</i> , Peripheral entoderm
<i>Coel.</i> , Coelomic cavity	<i>Roof End.</i> , Roof entoderm
<i>Ect.</i> , Ectoderm	<i>S. C.</i> , Sex-cells
<i>Gut End.</i> , Gut entoderm	<i>S. Gl.</i> , Sex-gland
<i>Int.</i> , Intestine	<i>Sub-Germ. Cav.</i> , Sub-germinal cavity
<i>Lat. Mes.</i> , Lateral plate of mesoderm	<i>Sub-Germ. End.</i> , Sub-germinal entoderm
<i>Mes.</i> , Mesentery	<i>Sw. Bl.</i> , Swim bladder
<i>Meson.</i> , Mesonephros	<i>Vit. End.</i> , Vitelline entoderm
<i>Myo.</i> , Myotome	<i>Wolff. D.</i> , } Wolffian duct
<i>Noto.</i> , Notochord	<i>W. D.</i> , }
<i>P. Card.</i> , Post cardinal vein	

## PLATE 1

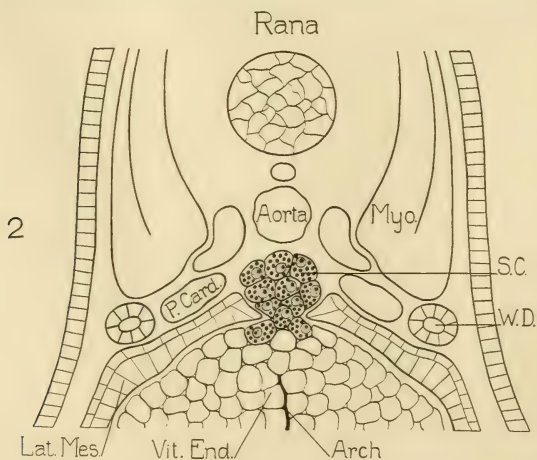
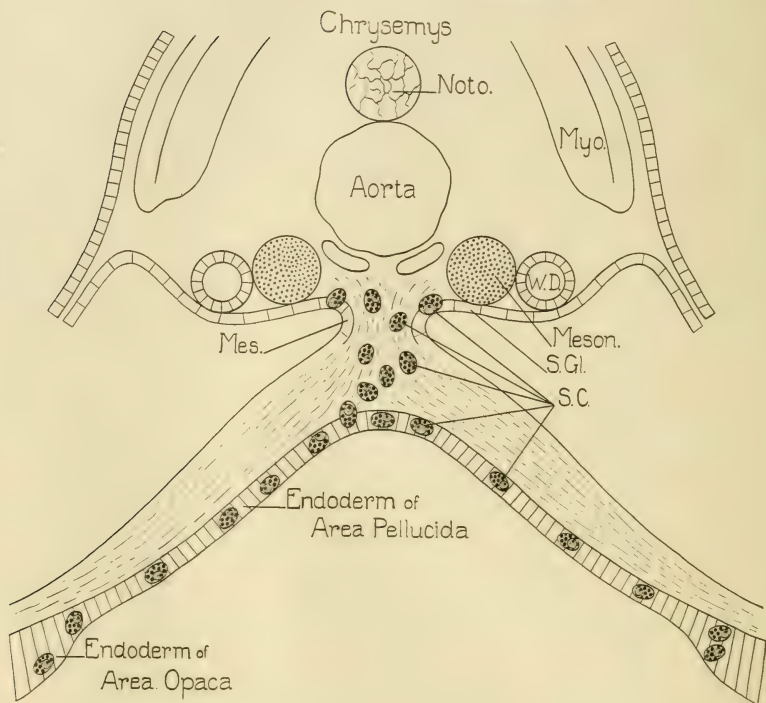
### EXPLANATION OF FIGURES

- 1 Diagram to show the migration path of the sex-cells in *Chrysemys marginata*.
- 2 Diagram to show the migration path of the sex-cells in *Rana pipiens*.

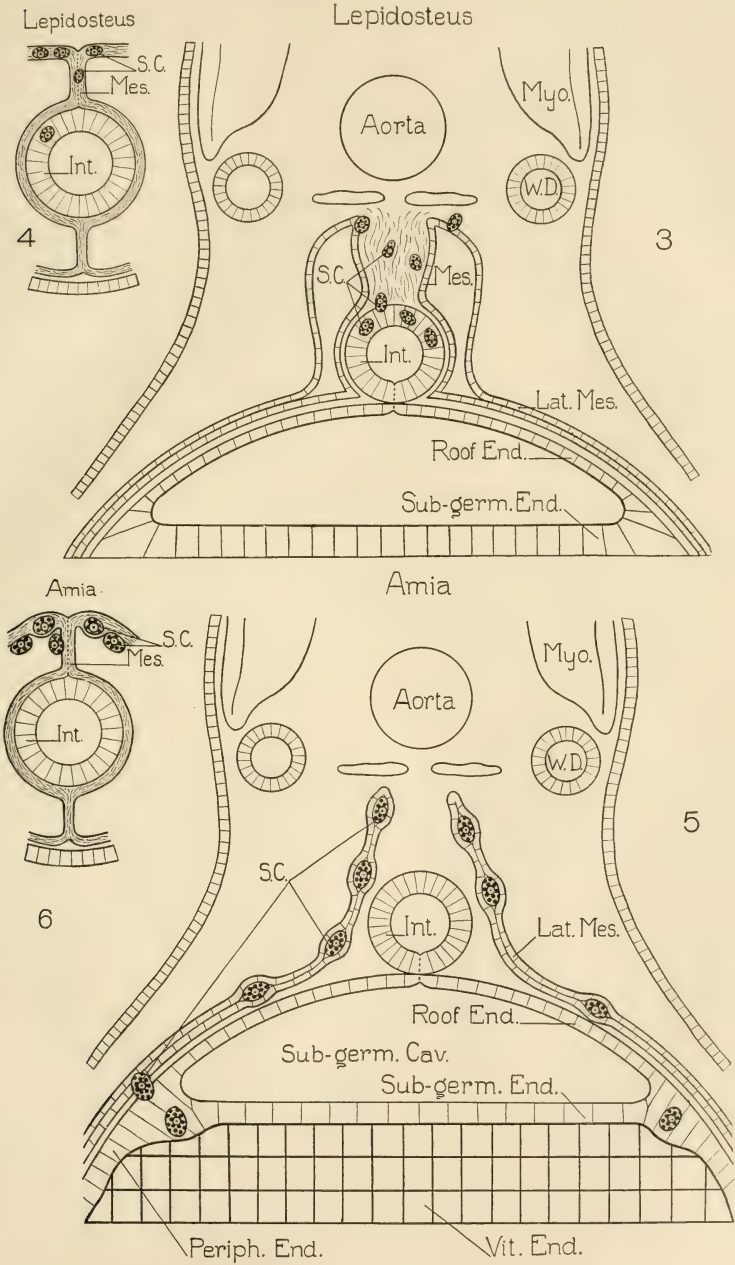
## PLATE 2

### EXPLANATION OF FIGURES

- 3 Diagram to show the migration path of the sex-cells in *Lepidosteus osseus*.
- 4 Diagram to show the last phase of the migration of the sex-cells in *Lepidosteus osseus*.
- 5 Diagram to show the migration path of the sex-cells of *Amia calva*.
- 6 Diagram to show the last phase of the migration of the sex-cells in *Amia calva*.



BENNET M. ALLEN



## PLATE 3

### EXPLANATION OF FIGURES

7 Transverse section through the hind-gut of an 8.6 mm. larva of *Lepidosteus osseus*.  $\times 300$ .

8 Transverse section through the hind-gut of a 9.3 mm. larva of *Lepidosteus osseus*.  $\times 300$ .

9 Transverse section through the hind-gut of a 10.7 mm. larva of *Lepidosteus osseus*.  $\times 300$ .

10 Transverse section through the hind-gut of a 14.1 mm. larva of *Lepidosteus osseus*.  $\times 300$ .

11 Transverse section through the hind-gut of a 17 mm. larva of *Lepidosteus osseus*.  $\times 300$ .



ERRATA

Gelatin plates 1, 2 and 3 should have been numbered 3, 4 and 5

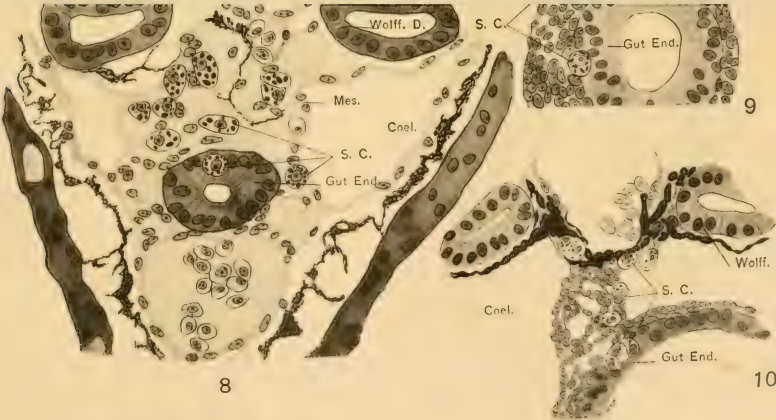
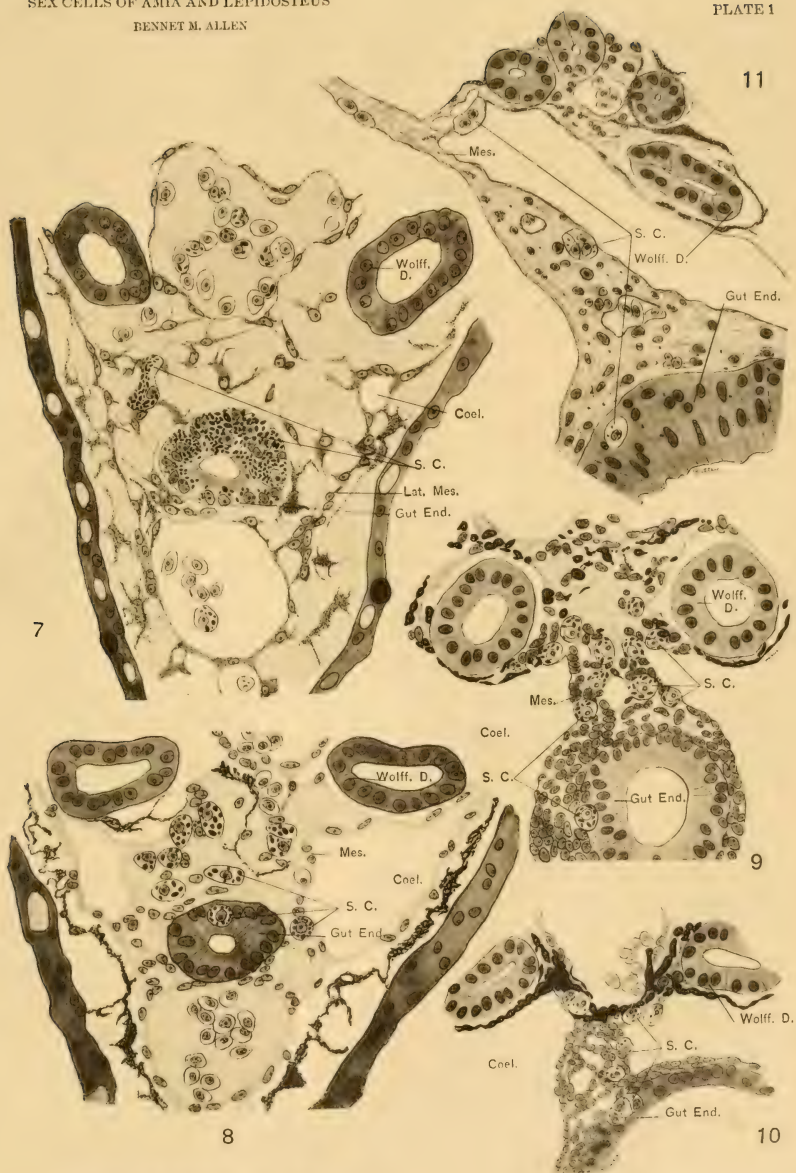


PLATE 3

EXPLANATION OF FIGURES

- 7 Transverse section through the hind-end of an 8.6 mm. larva of *Taxidea*







## PLATE 4

### EXPLANATION OF FIGURES

12 Transverse section of the rudimentary sex-glands of a 24 mm. larva of *Lepidosteus osseus*.  $\times 300$ .

13 Transverse section of a sex-gland of a 110 mm. specimen of *Lepidosteus osseus*.  $\times 300$ .

14 Part of a transverse section of a 17 mm. larva of *Lepidosteus osseus*, showing the reduced vitelline mass.

15 Detail drawing of a portion of the vitelline mass of the above section.  $\times 300$ .

16 Transverse section through the region immediately lateral to the posterior portion of the sub-germinal cavity of a 147 hr. embryo of *Amia calva*.  $\times 300$ . This shows the place of origin of the sex-cells.

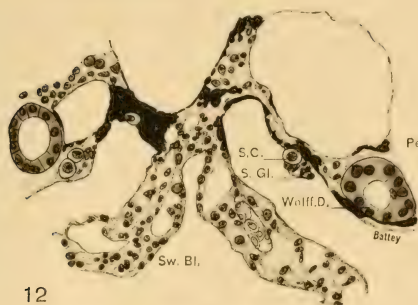
17 Section passing similarly through another specimen of the same stage of *Amia calva*.  $\times 300$ .

One sex-cell shown as it is pushing up into the mesoderm.

18 Transverse section through the hind-gut of a 6 mm. larva of *Amia calva*.  $\times 300$ .

19 Transverse section through the hind-gut of a 7 mm. larva of *Amia calva*.  $\times 300$ .

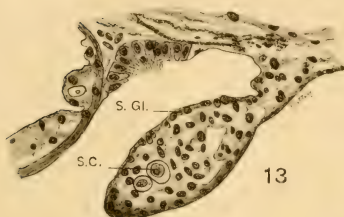
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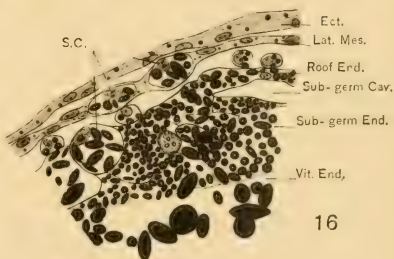
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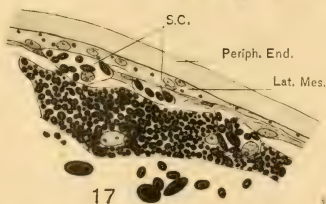
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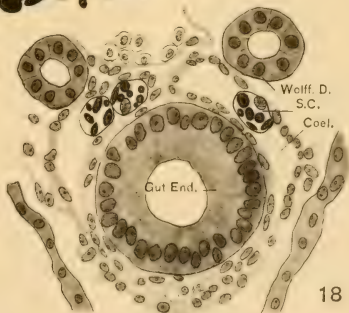
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17



19



18





## PLATE 5

### EXPLANATION OF FIGURES

20 Transverse section through the hind-gut of a 9.1 mm. larva of *Amia calva*.  
× 300.

21 Transverse section through the hind-gut and sex-gland anlage of an 11.4 mm. larva of *Amia calva*. × 300.

22 Transverse section through the young sex-glands of a 16 mm. larva of *Amia calva*. × 300.

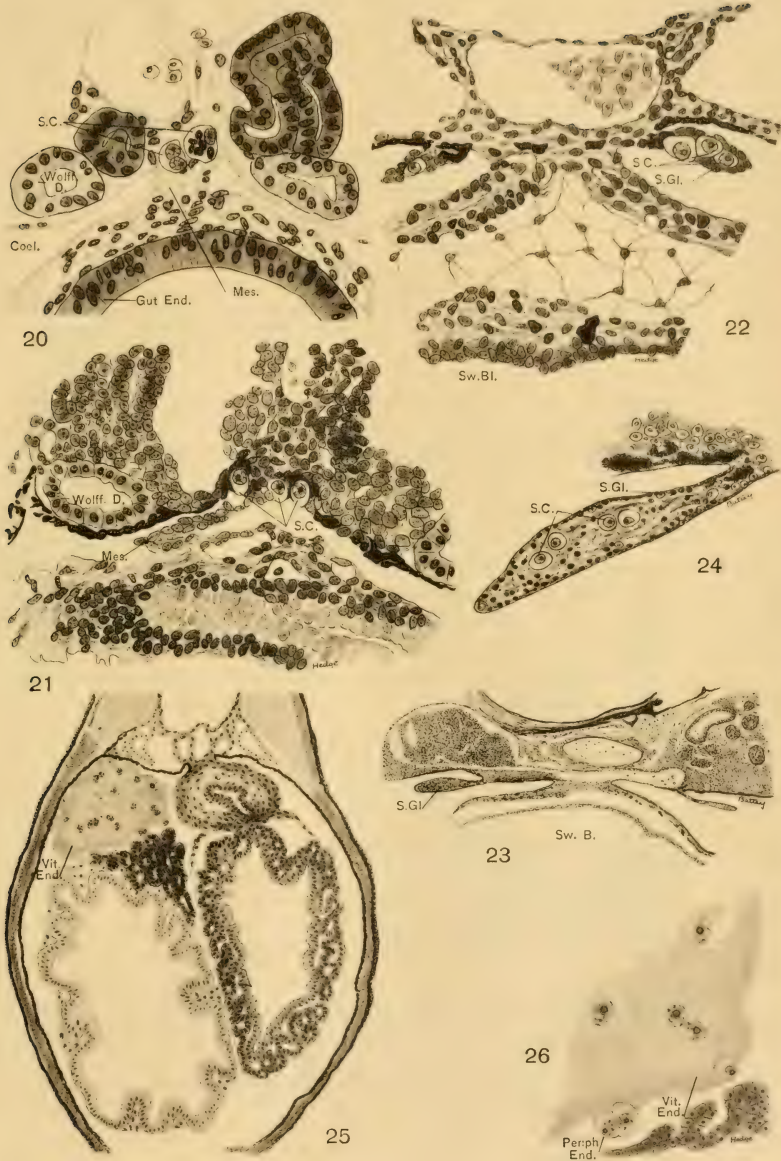
23 Sketch to show the orientation of the sex-glands in a 40 mm. specimen of *Amia calva* as seen in transverse section.

24 Detail drawing of the sex-gland as seen in above sketch. × 300.

25 Drawing to show the orientation of the much reduced vitelline mass of an 11.4 mm. larva of *Amia calva*.

26 Detail drawing of a portion of the vitelline mass indicated above. This shows the resemblance that certain cells of the peripheral entoderm show to sex-cells of this stage. × 300.

BENNET M. ALLEN





# THE CYCLIC CHANGES IN THE OVARY OF THE GUINEA PIG

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In the course of an experimental investigation into the causes of the cyclic changes taking place in the uterine mucosa and into the factors underlying the formation of the maternal placenta in mammals, we observed that cyclic changes in the structure of the ovary correspond to the uterine cycle. It has of course been known that at certain times ovulation takes place in the mammalian ovary, and furthermore, changes have been described as occurring in the ovarian follicles of certain mammals in connection with copulation and during pregnancy; but the cyclic changes taking place in the ovary quite independently of copulation and of pregnancy and merely dependent upon ovulation have, as far as we are aware, not yet been recognized. While we know of no publication dealing with the cyclic changes in the ovaries in general, a valuable study of the changes taking place during pregnancy in two species of Insectivores and in one species of Lemurid has been made by C. H. Stratz.<sup>1</sup> This author comes to the conclusion that in the period following copulation all the ovarian follicles become atretic; that during pregnancy small follicles are formed but also become atretic before they can develop; that only towards the end of pregnancy the follicles begin to grow to a considerable size, and that they reach the stage of maturity during the puerperium.

Stratz was not in a position to determine in an exact manner the time elapsed since the last copulation of the animals the ova-

<sup>1</sup> C. H. Stratz: der geschlechtsreife Säugethiereierstock. Haag. 1898.

ries of which he examined. He also seems to have examined a relatively very limited number of ovaries of animals during the different stages of pregnancy, and furthermore he studied only certain parts of each ovary. A methodical study of ovaries of non-pregnant animals was not undertaken. While his observation that after copulation all follicles become atretic is approximately, but not altogether correct, as far as its general validity is concerned, in the guinea pig the processes taking place in the ovaries during the subsequent stages differ from the conditions described by Stratz in the case of *Tupaja*, *Sorex* and *Tarsius*.

Furthermore Stratz does not recognize the essential factor upon which the cyclic changes in the ovaries depend. The conclusions in the last chapter of his publication show this clearly.

He summarizes as follows: If we find all follicles atretic, the animal has been pregnant. If at the same time a new corpus luteum is present, we have to deal with an early stage of pregnancy. If we detect some normal follicles, besides numerous atretic follicles and a new corpus luteum, we have to consider a puerperal condition of the animal. A large number of atretic besides a few normal follicles also suggests a puerperal state.

These general conclusions are not justified; the changes of the follicles do not, as Stratz assumes, depend upon pregnancy, and if we should attempt to use the criteria given by Stratz in the case of guinea pigs and mammals in general we would be liable frequently to make mistaken diagnoses. Notwithstanding, these necessary criticisms, the work of Stratz is very valuable and it advanced to a considerable extent our knowledge of the ovaries.

Since his publication no more detailed investigation into the processes taking place in the ovaries under various conditions has appeared, as far as we are aware. Within recent years, however, the question has been raised whether a new ovulation can take place during pregnancy.

We limited our investigations to the study of the ovary of the guinea pig. We examined several hundred pairs of ovaries of animals in which the period of the sexual cycle at which the ovaries

were obtained had been ascertained. In each case the entire ovary was cut into serial sections.

During the progress of our work new problems arose and an accident made it impossible for us to re-examine all our material in order to answer several questions which were raised at a later stage of our investigation. We especially regret our inability to determine the existence of follicles which were ready to rupture, in certain cases in which these data would have been of considerable interest. Our work is therefore incomplete in some respects. We expect, however, very soon to be able to supplement our present work, wherever necessary.

#### OVARIES OF GUINEA PIGS IN THE LAST STAGE OF PREGNANCY

The condition of the ovaries of a guinea pig in the last days of pregnancy is as follows: there are small, medium sized and large follicles without degeneration of granulosa cells. In other large follicles various stages of granulosa degeneration are present. Many follicles show further advanced stages of atresia, in which connective tissue grows into the follicular cavity. Especially numerous are the last stages of atresia in which the zona pellucida is directly surrounded by very cellular connective tissue. Mitoses are seen in the granulosa cells of the well preserved follicles. We also find here a few mature follicles which are characterized by an increase in cytoplasm of the granulosa cells. These follicles are large; their cavity is very wide. The nuclei of the granulosa cells are not as densely packed in these follicles as in the ordinary large follicles, this peculiarity being due to the marked development of the cytoplasm. They can be easily recognized in sections stained by haemotoxylin and eosin, inasmuch as they appear stained more reddish, in contradistinction to the ordinary large follicles in which the blue color of the nuclei predominates, while in the mature follicles the red stain of the cytoplasm is a distinguishing feature. In these mature follicles the number of mitoses is very much smaller than in the ordinary large follicles. With the increase in the quantity of cytoplasm and the relative decrease in the nuclear material,

the cell proliferation is diminished. The number of mitoses is usually very small, or mitoses may be absent in such follicles. Another characteristic feature is the relative lack of degeneration of the granulosa in these follicles. While the ordinary large follicles degenerate in the large majority of cases, the granulosa cells becoming karyorrhectic, as soon as the follicle attains a certain size; the mature follicles are very much more resistant. The changes in the granulosa cells described above and which lead to the transformation of an ordinary large follicle into a mature red-staining follicle, and simultaneously to a decrease in cell proliferation of the granulosa and to a diminished karyorrhexis of the granulosa cells, probably produces a decrease in cell metabolism, and this decrease in cell metabolism stands perhaps in a causal relation to the decrease in cell multiplication and to the greater resistance of the granulosa cell. A slight degree of degeneration of the granulosa may even occur in the mature red-staining follicles; a few of the central granulosa cells may degenerate; and in one case we observed even a fargoing degeneration of the granulosa in a mature follicle. It becomes therefore probable that these mature follicles also degenerate, if ovulation does not take place. This transformation of an ordinary large follicle into a mature follicle takes place only to a limited extent; the large majority of the follicles degenerate before they have reached the stage of full maturity. This holds good even in the case of guinea pigs before delivery, in which a rupture of follicles will soon take place.

The corpora lutea of pregnancy which, at the time at which we examined the ovaries, were approximately fifty-six to sixty-four days old and which had formed soon after copulation, show already some retrogressive changes in the lutein cells. A considerable number of the vessels entering the corpora lutea have a very thick wall consisting of several rows of cells. A large number of the vessels, however, have merely an endothelial lining. In many of the vessels no lumen is visible, the circulation through the corpus luteum being evidently not very active; some of the capillary vessels have, however, a widely open lumen. The quantity of the connective tissue in the centre of the corpus luteum is small,

on account of the previous proliferation of lutein cells which encroached more and more upon the space originally filled by the connective tissue. The corpora lutea are large. The lutein cells show signs of degeneration; they are finely vacuolar and may have a foamy appearance; a certain number of cells take less eosin and appear therefore pale. Many cells have a sharply defined, red-staining outline. The nuclei also show changes; they are frequently deformed, indented; or they are round, vesicular, but stain less with haematoxylin; they appear somewhat karyolytic. Mitoses could not be seen in the lutein cells. The degree of retrogressive changes may vary in different corpora lutea even in the same ovary.

We see therefore that even before delivery and before a new ovulation has taken place, degenerative changes set in in the corpora lutea, and it accords with these retrogressive changes that mitoses are absent or at least very rare in such corpora lutea.

Besides these corpora lutea of pregnancy we may find in such ovaries 'yellow bodies' representing the last stage of retrogression of corpora lutea. In the corpora lutea which were transformed into such yellow bodies, degeneration must have set in approximately sixty to sixty-five days ago. These 'yellow bodies' have the following structure: In their centre and periphery we find hyaline connective tissue; between these two zones of hyaline connective tissue a relatively small number of degenerated large lutein cells is enclosed, in which, during the process of retrogression, a large amount of yellow pigment was produced.

#### OVARIES OF GUINEA PIGS WITHIN TWO DAYS AFTER DELIVERY

In the period directly following delivery the condition of the ovaries, as far as follicles and corpora lutea are concerned, is approximately the same as in the period preceding it. The growth and degeneration of the follicles still continue to take place, and in follicles in which the granulosa has completely or almost completely degenerated an ingrowth of connective tissue and complete atresia of the follicles occur. The retrogressive changes in the corpora lutea also progress, but at a slow rate, and on the whole the

corpora lutea are not very different from those found in the preceding period. This description holds good for instance for ovaries of a guinea pig extirpated ten minutes after complete delivery.

Soon after delivery (usually within a few hours) the guinea pig is ready for a new copulation and ovulation, and after ovulation changes take place in the follicles which will be described later.

The corpora lutea of the preceding pregnancy undergo no very marked changes within the next two days after delivery, although vacuolization of the lutein cells and degenerative changes in the nuclei show probably a slight advance; the lutein cells do not stain as well with eosin and appear pale. If copulation take place soon after delivery, a rupture of the mature follicles occurs within the succeeding six or ten hours; but if copulation be prevented by isolating the female, ovulation frequently occurs, but does not need to take place within thirty-six hours after delivery. In several cases in which an actual copulation was prevented, in which however the male was in contact with the female for a short time after delivery, the rupture of the follicles and the formation of new corpora lutea took place in the usual way. The changes in the new corpora lutea within the first two days after delivery are the same as those described in a previous paper.<sup>2</sup>

In three cases the lower part of the uterus or the vagina of guinea pigs were tied completely or incompletely towards the end of pregnancy. This procedure led to the death of the fetuses, followed by expulsion of the dead fetuses in a case in which the occlusion had been incomplete. In another case the animal was killed by chloroform six days after the application of the ligature, and the fetuses were found dead; furthermore autolysis of the placenta had set in. In these cases especially the periphery of the corpora lutea of the preceding pregnancy showed vacuolization of the lutein cells. The nuclei were shrunken or somewhat chromatolytic. Notwithstanding the degenerative changes visible in the corpora lutea, no new ovulation had taken place. From these and other observations it follows that delivery as such does not lead to far-

<sup>2</sup> The formation of the corpus luteum in the guinea pig. *Journal American Medical Association*, February 10, 1906.

going changes in the ovaries; that merely a slow progress takes place in changes which had set in before delivery. We furthermore see that without copulation a spontaneous ovulation does not need to take place after delivery, notwithstanding the degenerative changes in the corpora lutea; that ovulation can, however, occur without copulation, and this seems to be the rule, if the male had been in contact with the female for some time after delivery, a copulation having been made impossible during this period of contact.

#### OVARIES OF NON-PREGNANT GUINEA PIGS IN THE PERIOD DIRECTLY PRECEDING OVULATION

This description applies to ovaries of guinea pigs which had copulated a few hours previously, in which an ovulation had however not yet taken place—ovulation usually taking place approximately six to ten hours after copulation. In another case we examined the ovaries of a guinea pig that was ready for copulation ('in heat') in which, however, an actual copulation had been prevented by occluding the vagina by means of a strip of plaster.

The condition of the follicles in these ovaries was similar to the condition found in ovaries preceding and immediately following delivery; we find good follicles of small, medium and large size; mitoses are present in the granulosa of such follicles. The majority of the large follicles however show more or less degeneration of the granulosa, with the exception of the few large follicles which progressed to complete maturity; they showed the cytoplasmic changes described above. In these as well as in some other well preserved large follicles the theca interna appears somewhat hyperemic. We also find the various stages of connective tissue ingrowth and of the subsequent diminution in the size of the follicles ('connective tissue atresia') which we described in the case of the other ovaries. In this case we do not find corpora lutea of a preceding pregnancy, but corpora lutea of an ordinary ovarian period, not accompanied by pregnancy. These corpora lutea are much smaller than those of pregnancy; their lutein cells show vacuolization, indicating the beginning of retrogressive changes. Notwithstanding these retrogressive changes an occasional mitosis

can still be found in lutein cells. The corpora lutea of the second last ovulation have in the meantime been transformed into yellow bodies. Processes of degeneration have therefore set in in the corpora lutea of non pregnant as well as of pregnant guinea pigs before ovulation. These beginning degenerative changes do however not prevent the occurrence of a few mitoses in the corpora lutea of previously not copulated animals, while in the degenerating corpora lutea of pregnancy we have so far not been able to detect the presence of mitoses in lutein cells.

#### OVARIES OF GUINEA PIGS WITHIN THREE AND ONE HALF DAYS AFTER OVULATION

In connection with ovulation certain far reaching changes take place in the ovaries. All follicles, with exception of very small ones, degenerate. These changes set in with ovulation, or they may perhaps start somewhat earlier, namely, simultaneously with those processes that bring about ovulation. As we have pointed out above, the general degeneration of the follicular granulosa which we find directly after ovulation cannot yet be observed before ovulation. This sudden degenerative process is quite independent of copulation; we found that it can be produced through ovulation without a preceding copulation. We discovered experimental means through which we can produce a spontaneous ovulation without a preceding copulation. Such an ovulation is followed or accompanied by the same degeneration of the granulosa. Moreover, if we keep a number of female guinea pigs separated from the males and if we examine their ovaries after various periods of isolation, we find occasionally ovaries in which the rupture of follicles had taken place a few days before. In this case also the typical follicular degeneration takes place independently of a preceding copulation.

Six and a half hours after a preceding copulation the ovaries showed, besides the presence of newly ruptured follicles, the following changes in the follicles: All, with the exception of very small follicles, show granulosa degeneration; in the large majority of the follicles almost the whole granulosa is found in a process of degeneration. We also find follicles in the process of connective tissue

atresia. Similar conditions are found in other ovaries at the same period.

Twenty-two hours after copulation some granulosa cells are found degenerated even in small follicles, (follicles having a small cavity); these degenerated granulosa cells are dissolved.

Similar changes take place in ovaries of guinea pigs in which ovulation followed delivery. In a guinea pig in which copulation took place two hours after delivery and in which the ovaries were examined seventeen hours after copulation, only a few quite small follicles without granulosa degeneration were found; in the large and also in the medium sized follicles much granulosa degeneration had taken place, the central granulosa cells degenerating first. Almost no entirely good follicles were left. As soon as the interna becomes exposed, phagocytic cells (rounded off interna cells) penetrate into the follicular cavity and these cells take up debris of the granulosa. The degeneration of the granulosa cells is as usual followed by ingrowth of connective tissue.

In other ovaries the granulosa may be degenerated to a great extent, but some remnants may still be left. Especially the granulosa cells of the discus proligerus survive usually the rest of the granulosa. We find of course various stages of connective tissue atresia besides the degeneration of the granulosa. From these observations it follows that the onset of degeneration of the granulosa must be extremely rapid.

If we extirpate the corpora lutea, from two to eight days after copulation a new spontaneous rupture of follicles takes place in most cases approximately from thirteen to fifteen days after the previous copulation, even if the female had been kept entirely isolated during the whole period following the extirpation of the corpora lutea. This early spontaneous ovulation is accompanied by the same follicular degeneration which we described above.

It is an interesting problem, whether an artificially produced rupture of a follicle, with the subsequent development of a corpus luteum, is accompanied by the same acute follicular degeneration. Several years ago we made experiments in which we pricked or cut the surface of ovaries of guinea pigs which were either 'in heat,' without however having copulated, or which copulated a few hours previously, or which had in some cases copulated from three to six

days previously. In only one case did we find a young corpus luteum the origin of which could reasonably be attributed to the cutting of the ovary and to the artificial rupture of a follicle. In this case an animal had been used which showed the first symptoms characteristic for the period of heat. Three days after the cuts had been made the ovaries were examined. One young corpus luteum was found in the cortex of the ovary. Blood and connective tissue were found in the center of the corpus luteum; connective tissue and vessels grew into the corpus luteum, which was very small. In this ovary we found good follicles of small medium and large size; we also found large follicles with beginning and with further advanced granulosa degeneration, and with beginning ingrowth of connective tissue. In as much as in no case of spontaneous rupture the follicles were found in a similar condition at that period after the rupture, it is very probable that *we have in this case to deal with an artificial rupture of follicles and that such an artificial rupture of follicles is not accompanied by the rapid degeneration of the follicular granulosa.*

On the basis of our previous results we can easily understand, why in all probability we succeeded in one case only in causing an artificial rupture of a follicle. Such an experiment does not promise to be successful, unless we have the chance of opening a mature follicle, and such an opportunity exists only at periods of very short duration.

In these ovaries we find usually two or three generations of corpora lutea; namely:

1. The young corpora lutea, developing in the recently ruptured follicles. These corpora lutea we have described elsewhere in their development up to the sixth day.

2. Corpora lutea that had formed at the time of the preceding ovulation, which had not been followed by pregnancy in female guinea pigs which had been kept separated from males. These corpora lutea are therefore in all probability approximately nineteen to twenty-eight days old. They show signs of beginning retrogression. Their lutein cells are more or less vacuolar, especially in the periphery, where the vacuolization usually begins; gradually the vacuolization progresses to the central part. In the center of

the corpus luteum we find a relatively small amount of fibrous tissue. We not only find capillary vessels but also vessels the wall of which consists of two coats penetrating the corpus luteum.

These corpora lutea begin to shrink very soon, and three days after the new rupture they are usually smaller than immediately after the ovulation. Notwithstanding the degenerative processes which are apparent in these corpora lutea, it is not uncommon to find still mitoses in the lutein cells of such corpora lutea within the first twenty hours after the new rupture of follicles has taken place. At a later period mitoses were not seen in this series. The mitoses appear in the relatively well preserved, but they may be present even in somewhat vacuolar lutein cells. It is possible that occasionally mitoses occur also in endothelial cells of the capillaries.

3. The third generation is represented by yellow hyaline bodies. They are the remnants of corpora lutea that formed forty or more days ago.

If we examine ovaries of young guinea pigs, two and a half to three months old, we may find only the first, or the first and second generations of corpora lutea, but yellow bodies may be lacking.

We see therefore that preceding and following the rupture of new follicles in non-pregnant animals, processes of degeneration have begun in the corpora lutea of the preceding ovulation, and that notwithstanding such processes of degeneration, mitoses may occur in such corpora lutea for a short period following the new ovulation. These corpora lutea which are not accompanied by pregnancy are much smaller than the corpora lutea of pregnancy and they shrink more rapidly. The absolute diminution in size is more rapid than in the retrogressing corpora lutea of a preceding pregnancy. Concerning the relative rapidity of retrogression (the percentage decrease in size, the full size of the corpora lutea being taken as the standard), we cannot make any definite statement, not having carried out any measurements.

The mode of retrogression is the same in both ordinary corpora lutea and in those of pregnancy. The vacuolization begins in the periphery, where it becomes most marked, and from here it proceeds into the interior of the corpus luteum.

## OVARIES OF A GUINEA PIG APPROXIMATELY THREE TO FOUR DAYS AFTER ABORTION

In one case the ovaries of a guinea pig were examined which on examination had previously been found to be in a well developed stage of pregnancy, but which had aborted about three to four days previously. The four corpora lutea showed signs of degeneration. The lutein cells were vacuolar in the periphery, in the center the cells stained pale red with eosin, the vesicular nuclei showed a diminution in the amount of chromatin. The cell outlines were very sharp, staining red with eosin. In the center there was dense connective tissue and many blood vessels had very thick walls.

Follicles of small, medium and large size, with well preserved granulosa, were present. A few mature, red staining follicles without mitoses or degeneration in the granulosa were also found. Many other large immature follicles showed various stages of granulosa degeneration. There were of course also present various stages of connective tissue atresia.

We see therefore that abortion is not followed by or associated with marked changes in the follicles. Whether the mature follicles which we found in these ovaries matured as a result of abortion, or whether the mature follicles were present before the onset of abortion, we cannot state with certainty, although it is more probable that maturation of the follicles followed abortion. We also note the beginning retrogressive changes in the corpora lutea. But in this case also we cannot be sure that the degenerative processes had not set in before the abortion had commenced.

## OVARIES OF GUINEA PIGS FOUR TO SEVEN AND ONE HALF DAYS AFTER OVULATION

Six days after an ovulation we find in the ovaries on the whole the following condition of the follicles: There are well preserved follicles of small and medium size, with mitoses in the granulosa cells. A limited amount of granulosa degeneration is found only in rare instances. In such follicles mitoses are absent or their

number is decreased. Follicles in an advanced state of connective tissue atresia are frequent.

The character of the follicles at this period of the sexual cycle is the same in cases in which the last ovulation was preceded by delivery, in which, therefore, in the previous period of the sexual cycle a pregnancy was present, and in other cases in which the previous period of the sexual cycle had not been complicated by pregnancy. We see therefore that within six days quite small follicles, possessing only a very small follicular cavity, grow and reach medium size. During this period the granulosa of medium sized follicles did not degenerate, and no large follicles had as yet developed. We find therefore principally, besides the follicles with preserved granulosa, follicles in an advanced state of connective tissue atresia.

Six days after ovulation we find the corpora lutea of the last generation (corpora lutea six days old, as follows: The center of the corpus luteum is filled by a more or less loose connective tissue. Mitoses are present in the lutein cells as well as in the endothelial cells of the capillaries. Almost all the vessels have a capillary character. They penetrate into the central connective tissue. At that period vessels with two coats (intima and muscle coat of the media) can be observed for the first time, although they become more frequent at a somewhat later period.

In guinea pigs in which a pregnancy and delivery preceded the last ovulation, the corpus luteum of the preceding pregnancy shows marked signs of degeneration. Especially the peripheral cells are frequently coarsely, while the more centrally situated cells are more finely vacuolar; but even in the latter the protoplasm stains less with eosin and the nuclei are slightly chromatolytic; the cells appear distinctly pale. The vessels are very thick and at certain places in the periphery the connective tissue of the neighborhood seems to begin to grow into the peripheral parts of the corpus luteum.

The ordinary corpora lutea of the second generation (not accompanied by pregnancy) show marked vacuolization; they diminish in size and in one case yellow pigment developed in a few of the vacuolar cells. Therefore in the course of five to eight days

since the beginning of degeneration the retrogressive changes have much advanced. The retrogressing corpora lutea of pregnancy of the corresponding generation are much larger at this period than the ordinary corpora lutea.

In a certain number of ovaries we also find a further (third) generation of retrogressing corpora lutea, represented by yellow bodies.

One corpus luteum deserves especial mention. In an ovary of a guinea pig which had ovulated approximately four and a half days before, five corpora lutea were found, four of which showing the typical structure. In the fifth of these corpora lutea, however, the lutein cells were arranged in the shape of glandular ducts. This condition has perhaps been produced through a dissolution of the central cells. Otherwise the corpora lutea in this ovary were normal.

The same typical changes in the follicles noticed in ovaries of this period after a preceding copulation and ovulation are also found in ovaries in which a spontaneous ovulation took place independently of a preceding copulation. As we stated above, such a spontaneous ovulation can be produced through an early excision of the corpora lutea. The same follicular changes take place also in pregnant animals in which, through an excision of the corpora lutea about six to eight days after copulation, a spontaneous ovulation is produced approximately thirteen to fifteen days after the beginning of pregnancy, without the pregnancy being interrupted.

We see therefore that these cyclical changes in the ovaries are essentially independent of copulation and of pregnancy and are directly connected only with ovulation.

#### OVARIES OF GUINEA PIGS SEVEN AND ONE HALF TO EIGHT AND ONE HALF DAYS AFTER OVULATION

At this stage of the sexual cycle we find good follicles of small, medium and large size with no, or only very little, granulosa degeneration. We also find follicles in connective tissue atresia. We see therefore that in approximately eight days follicles originally

very small have reached a large size. The new (eight days old) corpora lutea grow actively during this period and show frequent mitoses in lutein cells. The corpora lutea of the preceding ovulation (second generation) continues to shrink and show marked vacuolization of the lutein cells. If the second last ovulation were accompanied by pregnancy, the retrogressing corpora lutea were still larger.

The third generation of corpora lutea was represented by atretic yellow bodies the age of which varied approximately between forty-eight and ninety-five days.

#### OVARIES OF GUINEA PIGS TEN TO ELEVEN DAYS AFTER OVULATION

We find good follicles without granulosa degeneration of small, medium and large size, besides various stages of granulosa degeneration and of connective tissue atresia, early stages with beginning ingrowth of connective tissue included. In the granulosa of well preserved follicles mitoses are present as usual.

At this stage—ten days after ovulation—the ovary presents again its normal aspect. The follicles have grown to a large size and undergo the ordinary retrogressive changes. The ten to eleven days old corpora lutea are well developed; in the centre a relatively small amount of connective tissue is present. Mitoses in the lutein cells are usually frequent; they occur perhaps also in endothelial cells of capillaries. The large majority of the vessels have a capillary character, but occasionally a vessel is seen with a double coat of cells. Marked signs of degeneration are absent, but a few slightly vacuolar lutein cells may occasionally be seen.

The second generation of corpora lutea, originating in the second last ovulation, are small vacuolar bodies with much connective tissue and thick vessels. If, however, this second last ovulation had been followed by pregnancy, the retrogressing corpora lutea of the previous pregnancy are as yet much larger; the lutein cells have become very vacuolar; many thick vessels are present. In some of the vacuolar lutein cells yellow pigment appears.

A third generation of corpora lutea is represented by yellow bodies. They are, however, not found in all ovaries.

In this series of animals pregnancy had been prevented after a preceding copulation, either by ligaturing the tubes within the first two days after copulation, or by making long incisions into the uterus approximately four to six days after copulation.

The ovaries were also examined in a certain number of other guinea pigs of this period in which pregnancy existed. The accompanying pregnancy does not produce any marked change in the ovaries and the preceding description applies on the whole equally well to these ovaries.

#### OVARIES OF GUINEA PIGS THIRTEEN TO FIFTEEN DAYS AFTER OVULATION

In this series of animals pregnancy was prevented in the same manner as in the series of animals examined ten to eleven days after ovulation. The follicles have approximately the same character as in the previous period. We see the same varieties of follicles. Small follicles grow and become large and, after having reached this stage, or even at a slightly earlier stage, granulosa degeneration sets in with consecutive connective tissue atresia. In the granulosa of well preserved follicles numerous mitoses are present, and mitoses may even be found, if a slight amount of granulosa degeneration has taken place. The corpora lutea of the last ovulation (I generation) show more generally the beginning of vacuolization, especially in the periphery of the corpus luteum; but on the whole the corpus luteum is still well preserved and usually mitoses are found in some of the lutein and occasionally in cells belonging to blood vessels.

In the center we find connective tissue with thin spindle-shaped nuclei, and a number of vessels with walls consisting of several rows of cells penetrate into the central connective tissue. In some of the lutein cells the protoplasm appears dense and stains deeply with eosin. It appears probable that in such cells the nucleus had started to divide by mitosis, but degenerative processes seem to have set in and interrupted the process of the mitotic division. We are however not certain that this interpretation,

which would perhaps agree with an opinion expressed by Regaud and Dubreuil,<sup>3</sup> is correct.

The second generation of corpora lutea is represented by small vacuolar bodies with relatively much connective tissue and thick vessels. These atretic corpora lutea originated at the time of the second last ovulation and are therefore approximately thirty-three to forty days old. If this second last ovulation had been followed by pregnancy, the corpora lutea of this period are still much larger than the corpora lutea of the corresponding generation without an accompanying pregnancy; but a considerable shrinking of these corpora lutea has also taken place. The vessels are to a great extent collapsed. The lutein cells are finely or coarsely vacuolar, take less stain, still possess nuclei and a distinct cell wall, staining with eosin. The third generation of corpora lutea is again represented by yellow bodies. They are not present in all ovaries, but are found especially in the ovaries of older guinea pigs. Occasionally the degenerating corpora lutea of the second generation may also be absent.

In guinea pigs in which the last ovulation was followed by pregnancy, the condition of the follicles is very similar. The corpora lutea of the first generation, however, are large and show frequent mitoses in lutein cells, occasionally also in lutein cells the periphery of which is vacuolar. There are possibly also mitoses present in the endothelial cells. The retrogressing corpora lutea of the second and third generations are in pregnant animals of a similar character as those described in the ovaries of guinea pigs of the same period without an accompanying pregnancy.

#### OVARIES OF GUINEA PIGS FIFTEEN TO NINETEEN DAYS AFTER OVULATION

Pregnancy had in most cases been prevented by the same means which were used in the preceding stages. In a few instances in which pregnancy had occurred an early abortion followed. The follicles exhibit on the whole the same character as in the preceding stage; we find good follicles of small, medium and large size,

<sup>3</sup> C. R. Soc. Biol., 54. 1908.

and follicles in various stages of granulosa degeneration and of connective tissues atresia. We may also find large mature follicles. In how many cases these latter are present, will still have to be determined. In such animals a rupture of follicles is imminent.

In three guinea pigs a spontaneous ovulation had taken place at this period, notwithstanding the absence of male guinea pigs. In such cases young corpora lutea were found and, accordingly, a condition of the follicles characteristic of a period directly following ovulation. In the large majority of cases however a spontaneous ovulation did not take place in ovaries at this period of the sexual cycle. In such cases the follicles showed the character described above.

The corpora lutea of the first generation, which originated as a result of the last ovulation, show more or less signs of beginning retrogressive changes as indicated by fine or coarse vacuolization of the lutein cells. The intensity of this degenerative change varies in different ovaries. On the whole the retrogressive changes seem to be more marked in the nineteen days than in the sixteen days old corpora lutea; but variations seem to occur, even in corpora lutea of the same age. The vacuolization is usually most marked in the periphery and progresses toward the center. Other lutein cells are still more solid and mitoses in lutein cells can be seen in the majority of the corpora lutea of this period. In cases in which mature follicles are present and a spontaneous rupture of follicles is therefore soon to be expected, the corpora lutea show much vacuolization; but here also mitoses are still present in lutein cells.

In some cases the retrogressive changes are still further advanced and a connective tissue capsule may appear in the periphery of the corpus luteum. The marked vacuolization of peripheral lutein cells may be accompanied by a diminution in the lumen of blood vessels. Vessels with coats consisting of several rows of cells are seen regularly in these corpora lutea. The connective tissue in the center of the corpora lutea is usually dense and relatively small in amount.

In those cases in which a new spontaneous ovulation had taken place the vacuolization of the corpora lutea had still further pro-

gressed and under such circumstances mitoses were no longer present in them.

The corpora lutea of the preceding (II) generation, originating in an ovulation that took place at least thirty-seven days ago, are sometimes represented by small bodies which are surrounded by a thick connective tissue capsule; much fibrous tissue is found in the center and the lutein cells between these two zones show very large vacuoles. The vessels remaining in such structures have very thick cellular walls. In other cases some yellow pigment appears in such vacuolar cells and in still other cases we see only yellow atretic bodies. It is probable that the latter structures are found in cases in which a still longer time has elapsed since the preceding (second last) ovulation. There may of course have occurred a longer interval than twenty days between the last and second last ovulation.

When the second generation was represented by a corpus luteum of pregnancy, the retrogressive changes were also marked, shrinking of the corpus luteum and vacuolization of the lutein cells are pronounced, but such corpora lutea are still considerably larger sixteen to nineteen days after the completion of pregnancy than ordinary corpora lutea of the corresponding generation. Some of the vacuolar cells may show a yellow pigmentation. In such ovaries we may find a still older generation of retrogressing corpora lutea present, represented by yellow atretic bodies which owe their origin to an ovulation that took place more than a hundred days ago; and if the last named (third last) ovulation were followed by a pregnancy, this ovulation may have taken place approximately one-hundred and fifty days ago. Not in all animals are so many generations of corpora lutea found; especially in young animals (two to three months old only one generation may be present.

If the last ovulation that took place fifteen and one half to nineteen days ago were followed by pregnancy, the follicles in the ovaries of pregnant animals of this period do not show any marked difference from the follicles of non-pregnant animals at the corresponding period after ovulation. In both cases we find good follicles of various sizes and the different stages of retrogression of

follicles which we mentioned above. In the ovaries of pregnant animals of this period we may also find mature follicles, the granulosa cells of which have more cytoplasm that stains red with eosin. Such follicles show less granulosa degeneration and a decrease in the number of mitoses is visible in the granulosa cells. Some degeneration of granulosa cells may however occur in these follicles and their further fate will still have to be determined.

The corpora lutea of pregnancy (first generation) are well preserved. Fine vacuoles may however be present, especially in the peripheral lutein cells. Mitoses are also present. They do not show such pronounced signs of retrogression, as occur in corpora lutea of non-pregnant animals of this period.

#### OVARIES OF GUINEA PIGS TWENTY TO TWENTY-SEVEN DAYS AFTER OVULATION

At this period the proportion of animals in which a spontaneous ovulation had taken place, notwithstanding the separation of females and males, is much greater than in the preceding period. Among twenty-two guinea pigs a spontaneous ovulation had taken place in eight, while in the fourteen other females no rupture of follicles had as yet occurred. In at least one and possibly in more of these fourteen guinea pigs a rupture was however imminent, as indicated by the presence of mature, red-staining follicles. In those animals in which ovulation had taken place within the last few days the follicles were in the condition corresponding to that stage after ovulation. The corpora lutea that originated as a result of the ovulation twenty to twenty-six days previously showed marked degeneration; the cells were vacuolar; in one case the lutein cells formed a hyaline material in which the vesicular nuclei were imbedded. Mitoses were present in only one case, in which the rupture had taken place apparently within the last twenty-four hours, but even vacuolar cells may divide mitotically. Many blood vessels have thick cellular coats and the blood vessels in general do not seem to be patent.

In all the other guinea pigs in which a new rupture of follicles had not yet taken place the follicles behave approximately in the same manner as in the previous stage; we see follicles of various

sizes without granulosa degeneration, and follicles of large and also of medium size in various stages of granulosa and connective tissue atresia.

In the ovaries of the guinea pig in which a spontaneous rupture of follicles was imminent, the twenty-two days old corpora lutea also showed the signs of early degeneration; some of the cells were still good, but the majority were vacuolar.

In the guinea pigs, in which a spontaneous ovulation had not yet taken place, the corpora lutea of the last ovulation were also in a process of degeneration, which was especially marked during the later stages, twenty-four to twenty-six days after ovulation; here the vacuolization was very pronounced, and occasionally connective tissue began to grow into the periphery of the corpus luteum. The vessels of these corpora lutea were very thick. In some other ovaries, especially in those examined twenty and twenty-one days after ovulation, the number of relatively well preserved cells was still greater. On the whole the number of mitoses found in lutein cells at this period is distinctly diminished.

The older generations of corpora lutea are represented by atretic yellow bodies, which are however not present in all animals. In one case a corpus luteum was present that originated as a result of an ovulation that took place approximately ninety-three days before and was accompanied by pregnancy. In this case twenty-seven days after delivery very little of the lutein tissue was left, the blood vessels had very thick coats, and the fibrous tissue of the remnant of the corpus luteum was very prominent.

If the ovulation which took place twenty to twenty-seven days before were followed by a pregnancy, no new spontaneous ovulation took place. The conditions of the follicles was the same as in those guinea pigs in which the last ovulation was not followed by pregnancy and in which no new spontaneous ovulation had as yet taken place. The corpora lutea of pregnancy of this period showed much less vacuolization, although a slight amount of it may have been present, especially in the periphery of the corpus luteum. Mitoses were more common in these corpora lutea of pregnancy than in the ordinary corpora lutea of the same period. Their size was also greater.

In regard to the ordinary corpora lutea and the corpora lutea of pregnancy of previous generations, the same retrogressive changes which were described above in the ovaries of non-pregnant guinea pigs of this period, were found in pregnant animals.

We see therefore that the condition of the corpora lutea indicates the condition of the follicles, and conversely the condition of the follicles indicates the history of the corpora lutea. At a certain time (approximately ten days) after the ovulation a certain equilibrium is reached between the growth and the degeneration of the follicles. Whether a quantitatively exact equilibrium is reached, cannot yet be stated. In proportion to the length of time which elapsed since the last ovulation, the probability of a new spontaneous rupture, with the subsequent changes in the follicles, becomes greater. At this and the preceding period signs of degeneration are present in the ordinary corpora lutea, which become the more marked the older the corpus luteum; the number of mitoses in lutein cells decreases with advancing age; they may however still be present in corpora lutea immediately following a new ovulation; the latter however is soon followed by further progressing degeneration of the corpus luteum of the preceding ovulation. If the ovulation that took place twenty to twenty-six days previously was accompanied by pregnancy, no new spontaneous rupture of follicles took place, the proliferation of the lutein cells continued, and degenerative processes in the corpora lutea were retarded.

Approximately twenty-five days after the completion of pregnancy the corpora lutea of pregnancy (second generation) have become small vacuolar bodies with thick vessels and fibrous tissue, while corresponding ordinary corpora lutea have at this time apparently been transformed into yellow bodies.

## OVARIES OF GUINEA PIGS TWENTY-SIX TO FORTY DAYS AFTER OVULATION

In five animals in which, in order to prevent pregnancy, both (and in one case one) of the Fallopian tubes had been ligated within twenty-six hours after copulation, and in which at a later operation incisions had been made into the uterus, no new ovulation had taken place at the time of the examination, twenty-six to thirty-four days after copulation. The corpora lutea (twenty-six to thirty-four days old) showed very marked retrogression; they were very vacuolar; their size was always diminished especially after thirty-two to thirty-four days, but differed somewhat in individual cases. Some corpora lutea formed small bodies containing very dense fibrous tissue in the center and enclosing in the periphery a relatively small number of very vacuolar cells. Other corpora lutea were still somewhat larger and contained a few better preserved cells.

Besides the retrogressing vacuolar corpora lutea some atretic yellow bodies could be found in some cases; they were remnants of corpora lutea at least fifty days old. In two other ovaries a spontaneous ovulation had taken place recently and the condition of the follicles was in accordance with the age of the new corpora lutea. Here also the thirty to thirty-two days old corpora lutea of the preceding ovulation were very vacuolar and contained blood vessels with a thick coat and much dense fibrous tissue.

## OVARIES OF A PREGNANT GUINEA PIG APPROXIMATELY THIRTY-FIVE TO FORTY DAYS AFTER COPULATION

In these ovaries we found good follicles of small, medium and large size without granulosa degeneration and with mitoses in granulosa cells; other follicles showed various stages of granulosa degeneration and of connective tissue atresia. Mitoses were absent or diminished in number in follicles in which granulosa degeneration existed.

In addition to the ordinary large follicles mature or almost mature follicles were seen in which the cytoplasm of the cells was well developed, and in which the granulosa contained only very

few mitoses which were found especially in the discus proligerus. Some of the nuclei of the granulosa cells appeared somewhat contracted in these follicles, but no marked degeneration of the granulosa cells was found.

The corpora lutea of pregnancy were large, the cytoplasm of the lutein cells stained red yellow with eosin; the cell outlines were quite distinct. The large majority of the lutein cells were compact and did not show vacuoles; the nuclei were vesicular. A few mitoses were found in lutein cells. Only very little connective tissue was present in the center of the corpora lutea. Some of the vessels had thick walls, while other vessels were of a capillary character and had either a wide or narrow lumen. We see therefore that also at later stages of pregnancy the follicles continue to grow and to degenerate, and that even at this period of pregnancy follicles may mature. The lutein cells of the corpora lutea of pregnancy continue to show mitotic nuclear figures and well preserved cytoplasm at a time when, in the ordinary corpora lutea, retrogression is very far advanced.

#### OVARIES OF GUINEA PIGS IN WHICH COPULATION HAD BEEN PREVENTED

A large number of ovaries were examined of female guinea pigs which had been kept separated from males for various lengths of time.

One set of guinea pigs was separated from males before sexual maturity had been reached. The ovaries were examined, when the animals were six and twelve months old. In every instance ovulation had taken place repeatedly and we usually found the three generations of corpora lutea which we described in the case of guinea pigs which had copulated, namely relatively young corpora lutea, retrogressing vacuolar corpora lutea and atretic yellow bodies.

In another series guinea pigs were guarded against contact with males after delivery, and were kept separated from males for various periods of time. In this case a spontaneous ovulation took place after delivery, at least in the majority of cases, even

without contact with males, and subsequently further ovulations occurred. Under such conditions the successive ovulations do however not occur in the same intervals in all animals; in some cases a delay in ovulation may take place: this accords well with our previous observations. The conditions of the follicles correspond to the time elapsed since the last ovulation, as indicated by the state of the corpora lutea.

Not in every case however does a spontaneous ovulation take place without contact with male. In several cases neither new nor retrogressing corpora lutea could be found in the ovaries of guinea pigs which, according to their age, ought to have ovulated, but in which no sign of heat had been noticed during an observation extending over a certain period of time. In other guinea pigs which had been in heat recently, but in which copulation had been prevented, no new ovulation corresponding to the period of heat had taken place at the time of examination.

#### SOME OBSERVATIONS ON THE POSTFETAL DEVELOPMENT OF THE OVARY OF THE GUINEA PIG

In connection with the cyclic changes in the adult ovary of the guinea pig, just described, we thought it of interest to determine the time at which these cyclic changes set in. For this purpose we studied a series of ovaries at different stages of the growing guinea pig.

1. *In the ovaries of a fetus near the time of birth* many follicles are present in the cortex. These follicles have not yet a cavity and the largest follicles have a granulosa consisting of three, or four rows of granulosa cells; in the latter some mitoses can be seen. No distinct differentiation appears in the connective tissue of the different parts of the ovary.

2. *In the ovaries of guinea pigs four, five and seven days old* we find a cavity in a certain number of the follicles; no atretic processes have as yet taken place. The theca interna cells are distinguished from the surrounding connective tissue through the

increase in the size of their nuclei. The connective tissue around the medullary canals is relatively dense. In the granulosa, theca interna and in the ordinary connective tissue stroma mitoses are frequent.

3. *The ovaries of guinea pigs eighteen days old* are larger; the follicles also have increased in size. Small and medium sized and in proportion to the as yet small size of the ovaries, relatively large follicles are present. In some of the follicles degenerative processes appear at this time, but the extent to which such changes have taken place differs in the ovaries of different animals. In the ovaries of some guinea pigs no degeneration of the granulosa has as yet taken place. In the ovaries of another guinea pig a few follicles showed a trace of granulosa degeneration, while in another follicle the granulosa degeneration was pronounced.

In the follicles of some ovaries we find even a beginning ingrowth of connective tissue into the follicular cavity, and in one case a cavity of a follicle was filled with loose connective tissue. The majority of the follicles are in a good condition; their cavity is larger than at the preceding stage and the interna is better developed and consists of more rows of cells. Mitoses are present in the theca interna and in the granulosa. The connective tissue between the follicles is a little more fibrous, and around certain blood and lymph vessels it is somewhat edematous and rarefied.

4. *In the ovaries of guinea pigs twenty-eight days old* the majority of follicles are in good condition and non-atretic; they are of small and medium, but not yet very large size. In some ovaries hardly any degeneration of follicles is visible; in others we see some follicles which have not yet attained their full size (corresponding to the as yet small size of the ovaries), presenting various stages of granulosa degeneration. In some follicles the granulosa has been entirely destroyed and connective tissue begins to grow into the cavity.<sup>6</sup> In some cases we find quite atretic connective tissue follicles. In some small and medium sized follicles the ova may undergo (probably amitotic) nuclear division and a corresponding segmentation of the cytoplasm, the granulosa being still intact.

In other cases, however, such ova are surrounded by connective tissue.

The connective tissue of the ovaries shows more differentiation at this period and is somewhat more fibrous.

5. *In ovaries of guinea pigs one to two months old* the size of some of the follicles, in correspondence with the growth of the ovaries, enlarges. We see various stages of granulosa degeneration and of connective tissue atresia. Granulosa degeneration may take place in medium sized and in large follicles. In some ovaries the large majority of follicles may show either granulosa degeneration or connective tissue atresia. Corpora lutea are not yet visible.

6. *Ovaries of guinea pigs three months old:* Approximately at this period the ovaries have become mature. We find various stages of developing follicles and occasionally mature follicles ready to rupture. We find the various stages of granulosa degeneration and of connective tissue atresia. We notice a greater differentiation in the structure of the stroma in different parts of the ovary.

Corpora lutea, which occasionally are already in the beginning of degeneration, are present in some ovaries; in other animals ovulation has not yet taken place.

It follows from these observations that degenerative processes in follicles set in approximately fourteen to eighteen days after birth, and ovulation and formation of corpora lutea appear in guinea pigs two to three and a half months old. The ovaries and follicles must have reached a certain size, before ovulation sets in. The time required for the development of small into large follicles, with subsequent beginning of degenerative processes, is somewhat longer in the young growing animal than in the mature guinea pig, but in both the periods of time are of a similar order (approximately nine and fourteen days respectively).

## SUMMARY

The principal result of our investigations we can state as follows:

In the ovary of the guinea pig (and probably of mammals generally) cyclic changes take place independently of copulation and of pregnancy.

A sexual period (the period between two ovulations) is accompanied by a series of changes in the follicles. As a result of the conditions leading to or accompanying ovulation the granulosa of all large and medium follicles undergoes a very rapid degeneration, which is very marked within an hour or two after ovulation, or perhaps even sooner. In the follicles in which the cavity is as yet very small, the degenerative processes are very slight or absent. These follicles do not seem to perish. These degenerative changes affect equally both ovaries of one animal, even if a rupture of follicles should have taken place in only one of the two ovaries. The local effect of the rupture of the follicle can therefore not be the cause of the follicular degeneration. Within the next few days the small follicles grow and gradually attain a large size. Eight days after ovulation large follicles are again noticeable. As soon as good sized and medium sized follicles have been formed they begin to undergo degenerative processes, the granulosa degenerating and becoming dissolved and connective tissue growing into the follicular cavity. This process ends in an almost complete disappearance of these follicles. In the meantime other follicles grow and, having reached a large size, they also degenerate. Thus after a first stage of general growth, comprising approximately ten days after ovulation, a certain equilibrium is reached in which new follicles are growing to a certain size, and in which other follicles of large or medium size degenerate. Whether certain quantitative differences in the proportion of the number of growing and degenerating follicles exist at different periods of this second part of the sexual cycle, will still have to be determined. This second period of equilibrium begins approximately ten days after the last ovulation, and it lasts until a new ovulation occurs. Gradually a few large follicles undergo still further changes, the cytoplasm of their granulosa cells enlarges, the number of mitoses in these

cells decreases and they become more resistant to those processes which lead to degeneration in other follicles. The follicles in which such changes have taken place are mature and ready to rupture. In the meantime the follicles that ruptured during the preceding ovulation developed into corpora lutea. The latter represent principally the hypertrophic granulosa cells of the ruptured follicles, which proliferate mitotically. After a certain stage of development has been reached, degenerative processes set in in the corpus luteum, which start in its periphery and proceed to the center. These degenerative processes set in very early, are noticeable eighteen to twenty days and are usually marked twenty to twenty-four days after the preceding ovulation. Throughout this period of beginning degeneration, however, some mitoses are still visible in certain lutein cells. At this period usually a new ovulation takes place. The exact time at which the new ovulation occurs varies however somewhat in different animals, ovulation occurring earlier in some animals than in others. In some cases it can be hastened through certain external factors, especially copulation, but in the large majority of cases it occurs sooner or later even without a preceding copulation.<sup>4</sup> After the new ovulation has taken place, the degenerative processes progress in the corpus luteum, although within the first twenty hours after ovulation mitoses may still be found in certain lutein cells. In the following period a considerable shrinking of the corpus luteum takes place; the connective tissue in the cortex and in the periphery becomes hyaline and forms a relatively prominent part enclosing a small number of very vacuolar cells. Gradually yellow pigment is deposited in these vacuolar cells and thus the corpora lutea become transformed into the atretic yellow bodies. The new ovulation was of course again followed by the typical changes in the follicles.

If the ovulation be followed by pregnancy, the *principal* changes taking place in the ovaries are on the whole the same. The only

<sup>4</sup> Whether or not in the guinea pig ovulation can take place independently of a preceding copulation has been a subject of controversy. Concerning the literature of this question see William H. Kirkham, *Biological Bulletin*, vol. 18, no. 5, April, 1910.

difference consists in a prolongation of the sexual cycle, which lasts as long as the pregnancy continues. The changes in the follicles are identical with those found in the ordinary sexual period not accompanied by pregnancy.

After copulation the period of growth following the sudden degeneration of the follicles is the same as in the ordinary sexual period, but the period of follicular equilibrium is much prolonged.

During this period of follicular equilibrium certain follicles can not only grow to a considerable size, but may even undergo the additional changes which indicate the maturation of the follicle. A rupture of follicles does not however take place during pregnancy under ordinary circumstances.

The corpus luteum of pregnancy differs from the ordinary corpus luteum mainly in its prolonged duration of growth and of life. At a time when, in the ordinary corpus luteum not accompanied by pregnancy, mitoses have ceased to be present and the retrogressive changes are very marked, mitoses are still seen in the corpus luteum of pregnancy. In the corpus luteum of pregnancy degenerative changes set in before the end of pregnancy has been reached, and they continue after delivery. A short time after delivery a new ovulation usually occurs, even if no copulation had taken place after delivery. The retrogression of the corpora lutea of pregnancy continues, but it requires much more time than the retrogression of an ordinary corpus luteum.

The mechanism that governs the sexual cycle in the ovary can be recognized only incompletely by observation and it has been the subject of an experimental investigation, the results of which we shall report in more detail elsewhere. We may however state that our experiments have shown that through extirpation of the corpora lutea the sexual cycle is shortened. The presence of well functioning corpora lutea inhibits a new ovulation. Pregnancy as such does not prevent ovulation. Ovulation can be made to take place even in pregnancy, if the corpora lutea be extirpated at an early period after copulation. And under such conditions the typical follicular changes follow the ovulation during pregnancy. As soon therefore as degenerative processes have set

in in the corpora lutea, either during pregnancy or outside of pregnancy, a new ovulation can take place. How far the presence of the corpora lutea influences the transformation of ordinary large follicles into mature follicles and how far its action merely concerns the rupture of the mature follicles, remains still to be determined.

It follows from our observations that the time of ovulation depends upon at least three different factors: (1) Changes taking place in the ovaries. It is necessary that mature follicles have been produced, before rupture can take place. Our experiments indicate that cuts into an ovary causing an opening of a follicle may possibly lead to the formation of a corpus luteum only at a time when mature follicles are present. A certain time must therefore have elapsed after ovulation before another ovulation can take place. During this period small follicles reach their full size. Thus a minimal time which must elapse between two ovulations is required. (2) The time at which the influence of the corpus luteum preventing ovulation ceases to be exerted. Our observations make it very probable that the retrogressive changes observed in the corpora lutea before ovulation indicate the necessary cessation of functional activity. It is however noteworthy that, notwithstanding such a cessation of activity, mitoses can still be observed in the lutein cells at this period. Whether the corpus luteum acts principally upon the last stage in the development of follicles (maturation) or merely upon the rupture of follicles will still have to be determined with certainty. We recall however the fact that we observed the occurrence of mature follicles during various stages of pregnancy, notwithstanding the existence of corpora lutea. (3) Certain more or less accidental conditions, as for instance copulation. It is probable that other circumstances also may accelerate or retard the rupture of the follicles. Such factors act probably indirectly by causing changes in the circulation in the ovaries. In the guinea pig these are not indispensable, but their place can be taken by other factors; or even the total absence of corpora lutea may in the guinea pig be sufficient to allow a new ovulation.

In the guinea pig ovulation occurs in the large majority of cases without any previous copulation. In many cases however copulation is not without significance even in the guinea pig; it *accelerates* ovulation. While, after delivery, a spontaneous rupture may take place without copulation, in other cases it does not occur without ovulation. Also in the ordinary period of heat ovulation does not need to take place without copulation. Copulation is therefore not without importance; but in almost all of these cases ovulation is only deferred and sooner or later it will take place without the male. So far as the literature has been accessible to us it appears that the role copulation plays had not been fully appreciated by former investigators. Certain observations which we made indicate that other factors besides a preceding copulation may influence ovulation, and we intend to continue our investigation in this direction.

Our observations enable us to give some data concerning the time relations in the growth of various ovarian structures.

*a Follicles.* In about six days after ovulation small follicles reach medium size. In approximately eight days large follicles have developed and now degenerative processes set in. Mitotic cell division is most pronounced in the granulosa before degenerative processes have commenced; but mitoses may still be seen, if a slight degree of degeneration exist.

*b Ordinary corpora lutea.* The development of corpora lutea within the first six days after ovulation has been described in a previous paper. At six days we see for the first time, besides the capillary vessels, blood vessels with walls consisting of two rows of cells penetrating into the corpus luteum; they become somewhat more frequent from the tenth day on. In the meantime mitotic division of lutein cells continues and the increase in these cells causes the central connective tissue to become smaller in amount.

In corpora lutea ten to eleven days old a few vacuolar cells are present in the periphery of the corpus luteum. From ten to fifteen days after ovulation vacuolization is still very slight in peripheral lutein cells. From fifteen to eighteen days more fine or coarse vacuolization may appear. Other lutein cells are still

more solid and mitoses are still present. If no new ovulation have taken place, degeneration becomes more marked after twenty days; twenty-four days after ovulation we noticed a small amount of connective tissue growing into the periphery. At this period the number of mitoses is already diminished. In cases in which, between the eighteenth and twenty-sixth day after ovulation, a new rupture of follicles sets in, the degenerative processes are still more marked; mitoses may still be seen in the course of the first day after rupture of the follicles, but they disappear afterwards and the degenerative processes progress. The vacuolization of the lutein cells increases, the corpora lutea shrink, the connective tissue becomes gradually hyaline and is relatively preponderating in quantity over the lutein cells. About six days after the new ovulation (in approximately twenty-six days old corpora lutea) yellow pigment may be seen for the first time in the vacuolar lutein cells. Eight days after the new ovulation the corpus luteum is much shrunken, and ten to eleven days after the new ovulation corpora lutea approximately thirty-one to thirty-two days old have been reduced to small vacuolar bodies, around which a strong connective tissue capsule may appear. Corpora lutea thirty-three to forty days old (twelve to nineteen days after new ovulation) still represent vacuolar bodies; but now gradually the transformation into a yellow body sets in. Corpora lutea about forty-five days old have the appearance of yellow bodies and they may probably persist as such for a long time, after the third ovulation has taken place. Thus three generations of corpora lutea may be present side by side in the same ovary.

*c. Corpus luteum of pregnancy.* The corpus luteum of pregnancy differs from the ordinary corpus luteum in the longer duration of mitotic division, and the delay in retrogressive changes. Although slight vacuolization may be noticeable at relatively early stages, the corpora lutea of pregnancy are still in a good condition thirty-five to forty days after ovulation and they may still show mitoses at this period. Towards the latter part of pregnancy however degenerative processes set in, vacuolization and loss in staining power of the nuclei, and other changes, are noticeable. Mitoses could not be seen at this stage, and they appeared to be absent

after delivery had taken place. From ten to twelve days after delivery yellow pigment was seen in a few of the lutein cells in the corpus luteum of the previous pregnancy.

Thirteen to twenty days after delivery the corpus luteum is still much larger than an ordinary corpus luteum at the same period after ovulation, but considerable shrinking has already taken place. Twenty-seven days after delivery the corpus luteum is very small and vacuolar, with much hyaline connective tissue, but has not yet been transformed into a yellow body; but at a later stage, approximately sixty days after delivery (or possibly somewhat earlier) the corpus luteum appears as a yellow body, and as such it may persist for some time.

d. In the *developing ovaries* degeneration of the granulosa and connective tissue atresia of follicles are found as soon as the follicles have reached a relatively large size; these retrogressive changes first appear in guinea pigs approximately fourteen to eighteen days old, while the first ovulation appears much later, namely two to three and a half months after birth.

# STUDIES ON CHROMOSOMES

## VII. A REVIEW OF THE CHROMOSOMES OF NEZARA; WITH SOME MORE GENERAL CONSIDERATIONS

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NINE FIGURES AND ONE PLATE

### CONTENTS

Introduction.....	71
Descriptive.....	73
1 The second spermatocyte-division in Nezara.....	73
a The idiochromosomes.....	73
b The double chromosome.....	77
2 The first spermatocyte-division.....	78
3 The growth-period and spermatocyte-prophases.....	80
4 The diploid chromosome-groups.....	83
General.....	84
5 The idiochromosomes.....	84
a Composition and origin of the XY-pair.....	85
b Modifications of the X-element.....	88
c Sex-limited heredity.....	94
d Secondary sexual characters.....	99
6 Modes in which the chromosome-number may change.....	99
Conclusion.....	105

### INTRODUCTION

In the first of these 'Studies' ('05a) I described the idiochromosomes (X and Y-chromosomes) of *Nezara hilaris* as being of equal size in the male, and reached the conclusion that in this species no visible dimorphism appears in the spermatid-nuclei. In my third 'Study' ('06), after examination of the female diploid groups, this species was assigned a unique position as the single then

known representative of a type in which a pair of idiochromosomes can be identified in both sexes, but are of equal size in both, and in which, accordingly, no visible sexual differences appear in the diploid nuclei. These conclusions, as is now apparent, were based upon a wrong identification of the idiochromosome-pair, which is not the smallest pair, as I then believed,<sup>1</sup> but one of the largest. When this fact was recognized, the true conditions soon became evident.

I was led to re-examine *Nezara hilaris* by the fact (very surprising to me) that in *Nezara viridula*, a southern species closely similar to *N. hilaris*, the idiochromosomes of the male are extremely unequal in size, and the dimorphism of the spermatid-nuclei is correspondingly marked. Upon returning to the study of *N. hilaris* it soon became manifest that the dimorphism is present in this species also, though in far less conspicuous form. The size-difference between the X- and Y-chromosomes is here often so slight that I did not at first distinguish it from an inconstant fluctuation of size, such as is sometimes seen between the members of the other chromosome-pairs. When, however, the identity of the XY-pair was correctly recognized, its constancy of position and of size in the second division enabled me to make an accurate comparison between it and the other bivalents; and this fully established the constant inequality of its members, which is constantly greater than that now and then seen in other pairs. Both species also exhibit some other very interesting features that I overlooked in my former studies.

*Nezara* can therefore no longer stand as a representative of the third of the types distinguished in my third 'Study,' but belongs with *Euschistus*, *Lygaeus*, etc., in the second type

<sup>1</sup> This was in part because in most of the other forms known at the time the idiochromosomes are in fact the smallest, or one of the smallest, pairs. In part, also, I followed Montgomery ('01) who described in this species two small "chromatin nucleoli" in the spermatogonial groups, and believed them to be identical with the chromatic nucleolus of the growth-period. In a later paper ('06) Montgomery states these "chromatin nucleoli" to be "apparently not quite equal in volume," and asserts that I was in error in describing them as equal. In my material they are certainly equal in the great majority of cases. However, this is not the idiochromosome-pair.

## DESCRIPTIVE

Since the two species agree very closely save in respect to the idiochromosomes they may conveniently be considered together. Before describing the divisions, attention may be called to a striking difference between the two species in respect to the size of the cells and karyokinetic figures. As a comparison of the figures will show, the spermatocytes and maturation division-figures of *N. hilaris* are much larger than those of *N. viridula*. In the spermatogonia this difference is also apparent, though less marked. In the ovaries, strange to say, it cannot certainly be detected, either in the dividing cells or in the nuclei of the follicle-cells or of the tip-cells at the upper end of the ovary. It would be interesting to make a more accurate study of these relations; but I will here only state that the differences between the two species seem to arise mainly through greater growth of the spermatocytes in *N. hilaris*. With this is correlated a greater size of the testis as a whole; but the size of the entire body in this species is but little larger, as far as I have observed, than in *N. viridula*.

As regards the general features of the divisions, the diploid groups of both sexes uniformly contain fourteen chromosomes, the first spermatocyte-division eight and the second seven, the idiochromosomes being, as is the rule in Hemiptera, separate and univalent in the first division.

### 1. *The second spermatocyte-division*

*a. The idiochromosomes.* Polar views of the second division always show 7 chromosomes which are usually grouped in an irregular ring of six with the seventh near its center (fig. 3 *j-m*, figs. 14, 15). In both species one chromosome of the outer ring (*s*) can usually be distinguished as the smallest, though this is not always evident owing to the apparent variations produced by different degrees of elongation. This is the chromosome that I formerly supposed to be the idiochromosome-bivalent, despite its peripheral position, and despite the fact, which I had myself described, that a similar small chromosome, also peripheral in posi-

## EXPLANATION OF TEXT FIGURES

Figures 1 to 9 are from camera drawings, and are not schematized except that in a few instances the chromosomes have been artificially spread out in a series in order to facilitate comparison. Figs. 2 *k-l* are somewhat more enlarged than the others. In all the figures *d* denotes the double chromosome or 'd-chromosome,' *s* the small chromosome, *X* the large idiochromosome and *Y* the small.



Fig. 1 The second spermatocyte-division in *Nezara viridula*. *a-d*, metaphases in side view; *e-g*, anaphases; *h, i*, polar views of two sister-groups, middle anaphase, from the same spindle and in the same section.

tion, appears in several other pentatomids (*e.g.*, in *Euschistus*, *Coen* and *Mineus*). But *Nezara* forms no exception to the rule that the central chromosome is the idiochromosome-bivalent. In *N. viridula* this is immediately apparent in side views (often also in polar views) where the central chromosome is seen to consist of two very unequal components, the smaller being not more than one fourth or one fifth the size of the larger (fig. 1 *a-c*). In the anaphases these separate and pass to opposite poles, while all the others divide equally (fig. 1 *e-g*). Polar views of middle or rather late anaphases, when both daughter-groups can be seen superposed in the same section, clearly show the marked difference of the two groups in respect to the idiochromosomes (fig. 1 *h-i*). All the facts are here so nearly similar to those seen in *Euschistus* or *Lygaeus* as to require no further description.

In *N. hilaris* the conditions differ only in that the two components of the central chromosome are but slightly unequal; but in the examination of at least two hundred of these divisions I have never failed to detect the inequality. A series of side views are shown in fig. 2 *a-i*, figs. 16-21, two of which show all the chromosomes. These figures illustrate practically all the variations that have been seen in the idiochromosomes. The most characteristic condition is that seen in 2 *a, b, d*, in which both idiochromosomes (X and Y) are more or less elongated and united end to end. Less often one of them assumes a more spheroidal form (fig. 2 *e, h, i*, fig. 17). The size-difference, though always evident, seems to vary slightly (perhaps because one or the other component may be more or less compressed laterally), but is always distinctly greater than that now and then seen in other bivalents.

Fig. 2 *j* shows a mid-anaphase<sup>2</sup> (cf. figs. 21-23) in which the inequality would hardly be noticed without close study and the comparison of other cases. Figs. 2 *k* and *l* are similar stages showing all the chromosomes spread out in a series for the sake of comparison. In both, the two idiochromosomes are easily distinguishable,<sup>3</sup> and the larger is seen to be *one of the three largest chromosomes*. Figs. 2 *m-n, o-p, q-r* and *s-t* are pairs of sister-groups, in each case from the same spindle in anaphase. All of these are selected from cases in which a distinct size-difference appears between X and Y, but there are also many cases in which this cannot be seen. Such a case was figured in fig. 4 *e-f* of my first 'Study' the correctness of which is confirmed by re-examination of the original section. This condition is due simply to the fact that the large idiochromosome is more elongated than the small, so that the size-difference cannot be seen in polar view; and for the same reason it is often not evident in polar views of the metaphase.

<sup>2</sup> This and the two following figures are a little more enlarged than the others.

<sup>3</sup> Fig. 2 *l* is the same group figured in fig. 4 *d* of my first 'Study,' carefully redrawn and corrected. A comparison of the two drawings will show that in the latter a distinct size-difference between X and Y is actually shown but is minimized by the fact that the former is represented a trifle too small, the latter a little too large. It is now also evident that they are connected by two connecting fibres instead of by one.

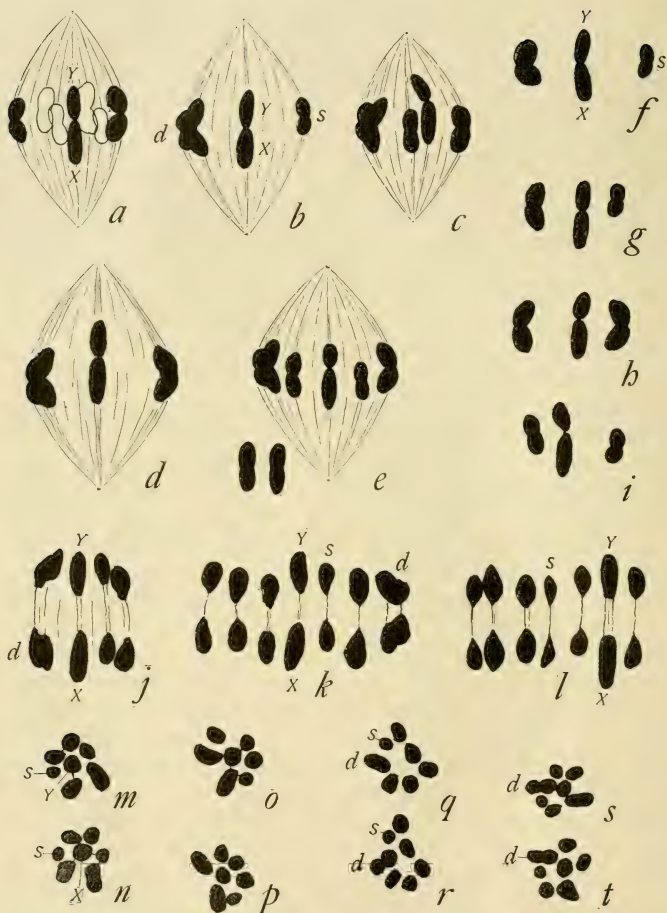


Fig. 2 The second spermatocyte-division in *Nezara hiliaris*. *a-i*, metaphase figures in side view, *a* and *e* showing all the chromosomes; *j-l*, mid-anaphases; in *k* and *l* all the chromosomes are shown artificially spread out in series; *m-n*, *o-p*, *q-r*, *s-t*, four pairs of sister-groups from late anaphases, in polar view, in each case from the same spindle.

*b. The double chromosome.* A second interesting feature of the second division that I formerly overlooked is the presence of a remarkable double chromosome which in the metaphase has exactly the appearance of a butterfly with widespread wings. This chromosome (which may be called the *d-chromosome*) is shown in profile view in 2 *b-e* and 1 *a-d*, 16, 17, 20, 24, 25. This is the only chromosome in the second division that shows any approach to a quadripartite form, and its characters are so marked as to constitute the most striking single feature of the division. As the figures show, it is one of the largest of all the chromosomes. It always has an asymmetrical tetrad shape, giving exactly the appearance of a smaller and a larger dyad in close union; and it always lies in the outer ring, so placed as to undergo an equal division, and with the larger wings of the butterfly turned towards the axis of the spindle. In polar view (3 *j-m*) the duality is far less apparent and sometimes invisible, even upon careful focussing. In *N. viridula* the duality is always apparent in side view, but the butterfly shape is usually less evident than in *N. hilaris*.

In the initial anaphases the *d*-chromosome divides symmetrically, drawing apart into two bipartite chromosomes (2 *j*, *k*, 1 *g*); but this is seldom evident save in profile view. Viewed from the pole the duality does not now ordinarily appear, though it may still sometimes be seen upon careful focussing. In the later anaphases the two components tend to fuse, and often can no longer be distinguished. Not seldom, however, the duality is visible even in the final anaphases; and sometimes this is so conspicuous that the spermatid-group seems at first sight to comprise eight instead of seven separate chromosomes (*n*, *r*, *s*, *t*).

Since the duality of this chromosome does not certainly appear in the spermatogonial groups or in the first spermatocyte-division, its peculiar form in the second division might be supposed to result from some special mechanical relation to the spindle-fibers in that division. This is, however, excluded by examination of the interkinesis, in which the chromosomes are irregularly scattered. In these stages, even when the spindle is still very small and the chromosomes lie in a quite irregular group, the butterfly shape is already perfectly evident; and it shows no constancy of

relation to the spindle-axis, often lying at right angles to the latter. Apparently therefore its duality arises quite independently of the spindle or astral rays, and its constant position in the fully formed spindle is the result of a later adjustment. In this species, as in many others, each chromosome is connected with the pole by a bundle of delicate fibers. In case of the *d*-chromosome this bundle is very broad, but I cannot be sure that it is double.

At first sight any observer would, I think, take the *d*-chromosome to be merely a result of the accidental superposition or close adhesion of two separate dyads of unequal size; but such an interpretation is inadmissible. When all the chromosomes can be unmistakably seen, the *d*-chromosome is found to constitute one of the seven separate elements invariably present in this division; and since the diploid number is 14 in both sexes this chromosome must represent one chromosome, not two, of the original spermatogonial groups. It is certain, therefore, that the double appearance does not result from close apposition of two separate chromosomes; it is therefore not a "tetrad" in the ordinary sense of the word—*i.e.*, not one that results from the synapsis of two chromosomes that are originally separate in the diploid groups.

## 2. *The first spermatocyte-division*

This division requires only brief mention. As stated, it shows eight separate chromosomes, of which the only one that can be positively identified is the Y-chromosome of *N. viridula*. This chromosome, always immediately recognizable in this species by its small size (3 *c*, *d*, *f*, *g*, *i*), figs. 12, 13), is usually central in position, like the *m*-chromosome of the Coreidae, but this is not invariable. Since it divides equally, and without association with any other chromosome (3 *g*) it is evident that the two idiochromosomes must be separate and univalent in this division. In *N. hilaris* (3 *a*, *b*, figs. 10, 11) the eight chromosomes usually form an irregular ring, there is no central chromosome, and neither idiochromosome can be certainly recognized. It nevertheless seems a safe inference from what is seen in *N. viridula* that the two idiochromosomes are here also separate and univalent.

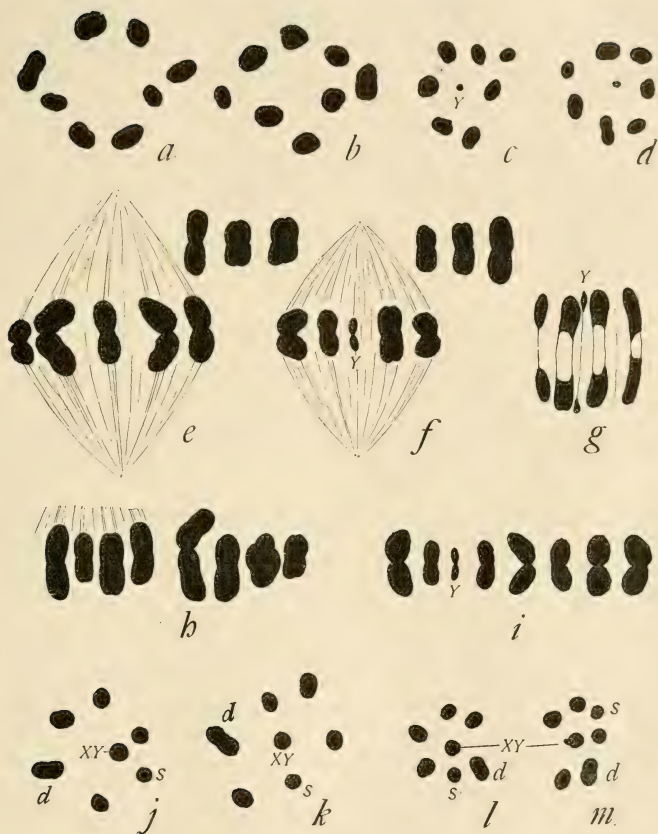


Fig. 3 First and second spermatocyte-divisions in the two species of *Nezara*. *a, b*, first division, hiliaris, polar views; *c, d*, corresponding views of viridula; first division, hiliaris, side view showing five of the chromosomes in position and the other three to the right above; *e, f*, corresponding view of viridula; *g*, middle anaphase, viridula, showing division of Y; *h*, first division metaphase, hiliaris, all the chromosomes artificially spread out in series; *i*, corresponding view of viridula; *j, k*, second division metaphase, hiliaris, polar views; *l, m*, corresponding views of viridula.

In this division the *d*-chromosome can not be identified in either species. Figs. 3 *e, f, h, i*, show all the chromosomes of the two species, in each case from a single spindle in side view. Most of them have a simple bipartite form, but in each species two or three of them often appear more or less distinctly quadripartite as is, of course, often the case with the bivalents in this division. In *N. hilaris* one of the largest chromosomes is usually more elongated than the others, and each half shows a slight transverse constriction. I suspect that this may be the *d*-chromosome, but cannot establish the identification.

### 3. *The growth-period and spermatocyte-prophases*

These stages fully bear out the conclusions based upon the divisions and establish the identity of the idiochromosome-pair with the chromatic nucleolus of the growth-period. Throughout the growth-period each nucleus contains a single intensely staining spheroidal chromatic nucleolus and in addition a very large, clearly defined pale plasmosome, which is sometimes double. Series of drawings of these two bodies (in each case from the same nucleus, and in their relative position) are given in figs. 4 *i-l* and *m-p*, from cells of the middle growth-period. They are also shown in figs. 26-29. In these stages no sign of duality is to be seen in the chromatic nucleolus, even after long extraction or in saffranin preparations. In later stages, as the chromosomes begin to condense, this nucleolus becomes less regular in outline, and gradually assumes a tetrad form, which becomes very clear as the chromosomes assume their final shape. This transformation may be traced without a break, successive stages being often seen within the same cyst. Just before the nuclear wall breaks down this tetrad is still clearly distinguishable from the others by its asymmetrical quadripartite form, as seen in 4 *y, z*, which show all the chromosomes (in each case from two successive sections). Figs. 4 *q-t* show four views of this tetrad at this period in *N. hilaris*, while *u-x* are corresponding views of *N. viridula*. These figures (which might be indefinitely multiplied) show the marked differences between the two species in respect to this tetrad, obviously corresponding to that seen between the idiochromosome

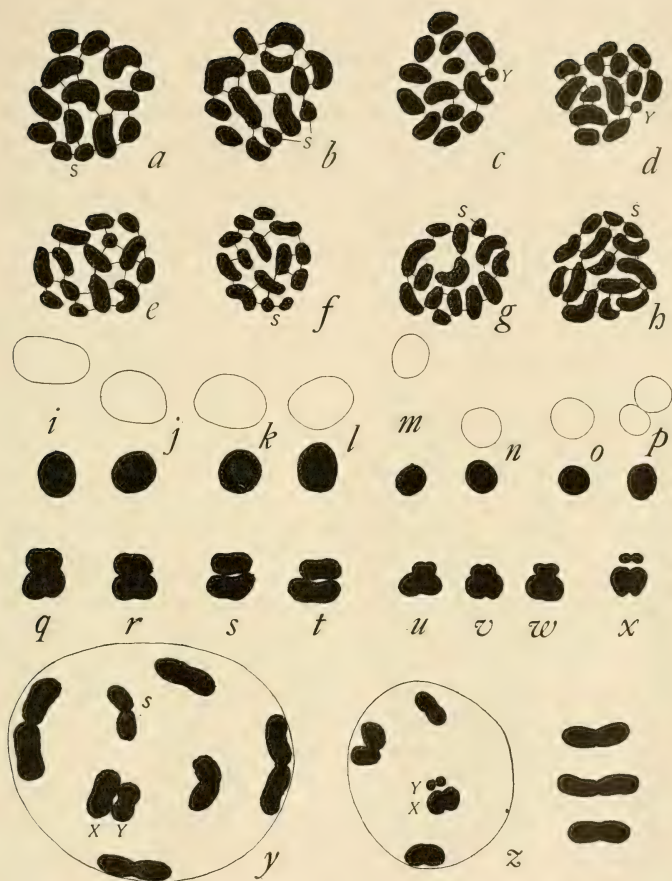


Fig. 4 The diploid groups, nucleoli of the growth-period, and late prophase-figures of the two species of *Nezara*. *a, b*, spermatogonial groups, hilaris; *c, d*, the same, viridula; *e, f*, ovarian groups, hilaris; *g, h*, the same, viridula; *i-l*, chromatic nucleolus and plasmosome from the growth-period, in each case from the same nucleolus in their relative position; *m-p*, corresponding views, viridula; *q-t*, the idiochrome-tetrad (chromatic nucleolus) from prophase nucleoli, hilaris; *u-x*, corresponding views, viridula; *y*, late prophase nucleus, showing all the chromosomes, hilaris (combination figure from two sections); *z*, corresponding stage, viridula, three of the chromosomes from adjoining section at the right.

bivalents of the two in the second division.<sup>4</sup> The two species may in fact readily be distinguished by mere inspection of the chromatic nucleolus at this period. Already at this time the two components are here and there seen to be separating, but as a rule they do not finally move apart until the nuclear wall has dissolved. From this time forward they cannot be individually identified with exception of the small idiochromosome of *N. viridula*, which is obvious at every period.

As far as my material shows, the earlier stages of the idiochromosomes can not be so readily traced in *Nezara* as in some other species, and the chromatic nucleolus can not actually be followed backward to the spermatogonial telophases—as can be done in such forms as *Lygaeus* or *Oncopeltus*, of which a detailed account will be given in a later publication. The prophase-figures, however, decisively establish its identity with an unequal pair of chromosomes that divide separately in the first spermatocyte-division; and in *N. viridula*, one of these is certainly the small idiochromosome. It may therefore confidently be concluded that the chromatic nucleolus is identical with the idiochromosome-pair, as in so many other cases. Comparison of the division-figures proves that this pair can not be identical with the small pair that I formerly supposed to be the idiochromosome-pair; and this small pair is moreover usually recognizable in the prophase groups (*s*, in 5 *y*, *z*) in addition to the unequal pair.

The foregoing facts make it clear that in *Nezara* the idiochromosomes undergo a process of synapsis at the same time with the other chromosome-pairs, and that their separation before the first division is a secondary process, to be followed by a second conjugation after this division is completed. A similar process often takes place in many other Hemiptera. There are, however, some forms, like *Oncopeltus*, in which the idiochromosomes are always separate, from the last spermatogonial division through all the succeeding stages up to the end of the first division. In this case, which I shall describe more fully hereafter, there can be no doubt that the conjugation which follows the first division is a primary synapsis, to be immediately followed by a disjunction.

<sup>4</sup> Cf. the earlier figures of the corresponding tetrad in *Brochymena* in my first 'Study,' fig. 7.

#### 4. *The diploid chromosome-groups*

In these groups the interest centers again in the identity of the idiochromosomes and the *d*-chromosome. Of the 14 separate chromosomes present in the diploid nuclei of both sexes, none shows any constant indication of duality (figs. 4 *a-h*). The *d*-chromosome can not, therefore, be identified in these stages. Secondly, in both species the diploid groups of the two sexes show the same relation as in other Hemiptera of this type, though this is, of course, more readily seen in *N. viridula* than in *hilaris*, owing to the small size of the Y-chromosome. In the spermatogonial groups of this species (4 *c, d*) this chromosome is at once recognizable while in the female groups (*g, h*) it is lacking, its place being taken by one of larger size. In both sexes the small pair (*s, s*) is also recognizable. In this species, accordingly, the Y-chromosome is confined to the male line, and the Y-class of spermatozoa must be male-producing, as in other forms.

In *N. hilaris* the Y-chromosome can not be identified (4 *a, b*), but the relation of the spermatozoa to sex-production is shown in another way, though less unmistakably than in *N. viridula*. As already described, the large idiochromosome or X-chromosome is one of the largest three chromosomes seen in the second division. We should therefore expect to see five largest chromosomes in the male diploid groups. This is clearly apparent in figs. 4 *a, b*, and is also shown in the corresponding figures of *N. viridula* (*c, d*) though not quite so clearly. One of these five in the male should be the X-chromosome; and if the usual relation of the spermatozoa to sex hold true, there should be six largest chromosomes in the diploid groups of the female. This relation actually appears in nearly all cases, and is shown in figs. 4 *e, f, g, h*, in each of which, again, the small pair (*s, s*) may be recognized. Though this evidence is in itself less convincing than that afforded by *N. viridula* (since the relation can not always be made out with certainty) it is fully in harmony with the latter, and sustains the same conclusion.<sup>5</sup>

<sup>5</sup> This relation is shown in my original figures of *N. hilaris*, though not quite as clearly as in the groups here figured. In my first 'Study' ('05) the five largest chromosomes are very clearly shown in fig. 4 *h*, and are also evident in 4 *g*. In the third 'Study' the relation is hardly evident in the male but fairly so in the female (figs. 5 *l, m*).

## GENERAL

5. *The idiochromosomes*

The case of *Nezara* shows how readily a morphological dimorphism of the spermatid-nuclei may be overlooked when the X- and Y-chromosomes do not differ markedly in size; and it emphasizes the necessity for the closest scrutiny of these chromosomes in the study of this general question. In my fourth 'Study' I placed with *Nezara hilaris*, as a second example of my original 'third type,' the lygaeid species *Oncopeltus fasciatus* (Dall.), on the strength of Montgomery's account of the conditions in the male ('01, '06) and my own unpublished observations on both sexes. While I have carefully re-examined this case also, I am not yet prepared to express an unqualified opinion in regard to it. Certainly, in very many of the cells of this species, at every period of the spermatogenesis, the idiochromosomes (which are always separate up to the second division) seem to be perfectly equal. A slight inequality may indeed be seen in some cases; but as far as I can yet determine this seems to fall within the range of the size-variation in other chromosomes and gives no positive ground for the recognition of a morphological dimorphism in the spermatozoa. A similar condition has been described in several other insects, notably in some of the Lepidoptera (Stevens, '06; Dederer, '08; Cook, '10), in the earwig *Anisolaba* (Randolph, '08) and apparently also in the beetle *Hydrophilus* according to Arnold ('08). I see no reason to question these observations; but the interpretation to be placed on them is by no means clear at present. The experimental evidence on the Lepidoptera seems to demonstrate that in at least one case—that of *Abraxas* according to Doncaster and Raynor,—it is the eggs and not the spermatozoa that are sexually dimorphic; that is, in the terms that I have recently suggested ('10a), in this case it is the female that is sexually 'digametic' while the male is 'homogametic.' Baltzer's careful work on the sea-urchins ('09) clearly demonstrates a cytological sexual dimorphism in the mature eggs of these animals, and shows that the spermatid-nuclei are all alike. In cases, therefore, where no visible dimorphism of the spermatid-nuclei is demonstrable, two possibilities

are to be considered, namely, (1) that it may be the female which (as in sea-urchins) is the digametic sex, and (2) that one sex or the other may still be physiologically digametic even though this condition is not visibly expressed in the chromosomes. The first of these possibilities may readily be tested by cytological examination of the female groups. The second can only be examined by means of experiment, and especially by experiments on sex-limited heredity. It is interesting that the work of Doncaster and Raynor, cited above, and the more recent one of Morgan on *Drosophila* ('10) have given exactly converse results, the former demonstrating a sexual dimorphism of the eggs, the latter of the spermatozoa. This agrees with the cytological data, as far as they have been worked out. The researches of Stevens ('08, '10), on the *Diptera* establish the cytological dimorphism of the spermatozoa in these animals, while all observers of the *Lepidoptera* have thus far failed to find such dimorphism in this group. It thus becomes a very interesting question whether a cytological dimorphism of the mature eggs may be demonstrable in the *Lepidoptera*; though a failure to find it would in no wise lessen the force of the experimental data. Physiological differences between the chromosomes are of course not necessarily accompanied by corresponding morphological ones—indeed such a correlation is probably exceptional.

(1) (a) *Composition and origin of the XY-pair.* The facts seen in *Nezara* again force upon our attention the puzzle of the Y-chromosome or 'small idiochromosome.' It is remarkable that two species so nearly akin as *N. hilaris* and *N. viridula* should differ so widely in respect to this chromosome; though this is hardly so surprising as the fact that in *Metapodius* this chromosome, as I have shown ('09, '10) may actually either be present or absent in different individuals of the same species. These facts show, as I have urged, that although the Y-chromosome shows a constant relation to sex when it is present, it can not be an essential factor in sex-production. As the case now stands this might be taken as a direct piece of evidence against the view that the idiochromosomes are concerned with sex-heredity. Further, as I have pointed out ('10) in *Metapodius* the introduction of super-

numerary Y-chromosomes into the female has no visible effect upon any of the characters of the animal, sexual or otherwise; and this might be urged against the whole conception of qualitative differences among the chromosomes and of their determinative action in development. It is especially in view of these and certain other general questions that I wish to indicate some of the many possibilities that must be taken into account in the consideration of this problem. My discussion is throughout based upon the *assumption* that the chromosomes do in fact play some definite role in determination, and that they exhibit qualitative differences in this respect. I do not hold that they are the exclusive factors of determination; though it is often convenient, for the sake of brevity, to speak of them as if they were such.

(2) Cytologically considered, the morphological dimorphism of the spermatozoa seems to have arisen by the transformation of what was originally a single pair of chromosomes comparable to the other synaptic pairs. We have at present no information as to whether the members of this pair were equal or unequal in size; but in either case there are grounds for the assumption that its two members differed in some definite way in respect to the quality of the chromatin of which they were composed. This pair, which may be called the primitive XY-pair, has undergone many modifications in different species, but without altering its essential relation to sex. In the insects (Hemiptera, Coleoptera, Diptera) its most frequent condition is that of an unequal pair, consisting of a 'large idiochromosome' or 'X-chromosome,' and a "small idiochromosome" or 'Y-chromosome,' the latter being confined to the male line, while the former appears in both sexes—single in the male and paired in the female. That all gradations exist between cases where X and Y are very unequal (as in many Coleoptera and Diptera and in some Hemiptera) and those in which they are nearly or quite equal (*Mineus*, *Nezara*, *Oncopeltus*) gives some ground for the conclusion that in the original type the XY-pair was but slightly if at all unequal.

By disappearance of the free Y-member of this pair has arisen the unpaired odd or 'accessory' chromosome, which accordingly

has no synaptic mate. This condition seems to have arisen in more than one way. It is almost certain that in many cases the Y-chromosome has disappeared by a process of gradual and progressive reduction (as indicated by the graded series observed in the Hemiptera (Wilson, '05b, '06). In some cases (of which *Metapodius* is an example) the same result may have been produced suddenly by a failure of the idiochromosomes to separate in the second spermatocyte-division (Wilson, '09b). A third possibility, first suggested by Stevens ('06), is that the X-element may have separated from a YY-pair with which it was originally united. This possibility seems to be supported by recent observations on *Ascaris megalocephala*, where the X-chromosome is sometimes fused with one of the other pairs, sometimes free (Edwards, '10).

(3) We have as yet no positive knowledge as to how the X-member of the XY-pair originally differed, or now differs, from the Y, or as to how this difference arose—a definite answer to these questions would probably give the solution of the essential problem of sex. There are, however, pretty definite grounds for the hypothesis that the X-member contains a specific 'X-chromatin' that is not present in the Y-member, and that the XY-pair is heterozygous in this respect. If this be so, the primary sexual differentiation is therefore traceable to a condition of plus or minus in this pair, accompanied by a corresponding difference between the nuclear constitution of the two sexes. (Cf. Wilson, '10a.) Further, there is also reason for regarding the heterozygous condition of this pair as due to the presence of the X-chromatin in one member of a pair which is (or originally was) homozygous in respect to its other constituents. The latter may be called collectively the 'Y-chromatin'; and we may, accordingly, think of the XY-pair as being essentially a YY-pair with one member of which the X-chromatin is associated.<sup>6</sup> Both the X-

<sup>6</sup> This suggestion is in principle the same as one earlier made by Stevens ('06, p. 54) that the Y-chromosome represents "some character or characters which are correlated with the sex-character in some species but not in others," with one member of which the X-chromosome is fused; and that "a pair of small chromosomes might be subtracted from the unequal pair, leaving an odd chromosome."

chromatin and the Y may themselves be composite, thus giving the possibility of many secondary modifications. The point of view thus afforded opens many possibilities for an understanding of sex-limited heredity, as indicated beyond.

(b) *Modifications of the X-element.* This view of the XY-pair is based upon two series of facts, of which the first includes the various modifications of the X-member of the pair seen in different species. It is, perhaps, most directly suggested by a study of the pentatomid species *Thyanta custator*.<sup>7</sup> In this common and widely distributed species I have found two races, which thus far can not be distinguished by competent systematists,<sup>7</sup> but which differ in a remarkable way in respect to both the total number of chromosomes and the XY-pair. In one of these races (which I will call the 'A form'), widely distributed throughout the south and west, the total number in both sexes is 16, and the XY-pair of the male is a typical unequal pair of idiochromosomes, exactly like that seen in many other pentatomids (*e.g.*, *Euschistus*, *Coenus* or *Banasa*). These are shown in fig. 5 *a, b*, their mode of distribution being the usual one. The second race (the 'B form') is thus far known from only a single locality in northern New Jersey. It differs so remarkably from the A form that I could not believe the observations to be trustworthy until repeated study of material, collected in four successive years, established the perfect constancy of the cytological conditions and the apparent external identity of the two forms. In this race the XY-pair is represented by three small chromosomes of equal size, which are always separate in the diploid groups and in the first spermatocyte-division (fig. 5 *i*), but in the second division are united to form a linear triad series (5 *c, d*). This group so divides that one component passes to one pole and two to the other (5 *e, h*), the

<sup>7</sup> I am indebted to Mr. E. P. Van Duzee for a careful study of my whole series of specimens of both races. He could find no constant differential between them. Additional studies of this material are now being made by Mr. H. G. Barber.

*Addendum.* Since this paper was sent to press Mr. Barber, after prolonged study, has reported his conclusion that the 'A form' is *Thyanta custator* of Fabricius, while the 'B form' is probably *Thyanta calceata* of Say, which has long been regarded as a synonym of former species.

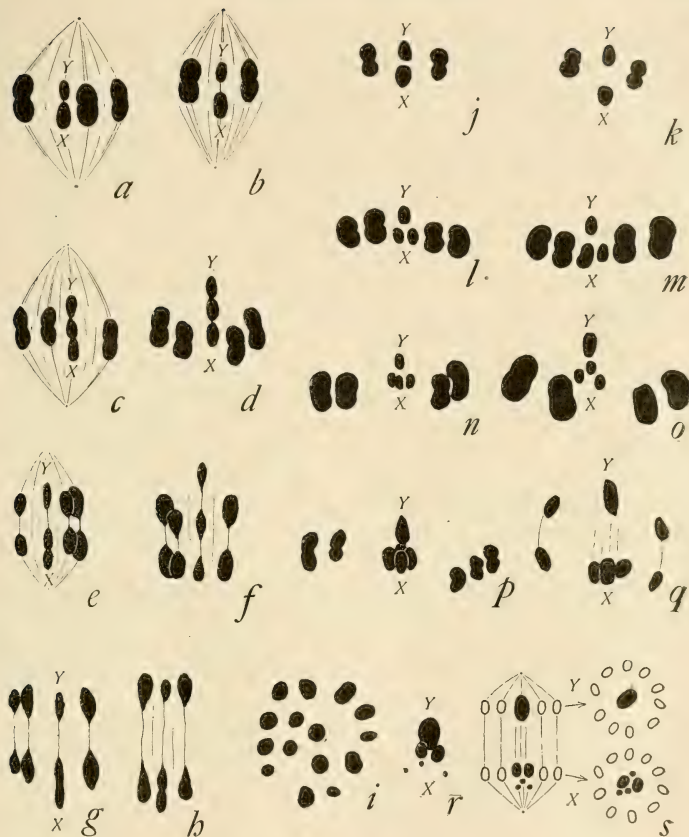


Fig. 5 Comparison of the XY-group in various Hemiptera. (*a-i* are original; the others from Payne.) *a, b*, *Thyanta eustator*, 'A form,' second division in side view; *c, d*, corresponding views of the 'B form'; *e-h*, anaphases of same; *i*, polar view of first division of same; *j, k*, metaphase chromosomes, second division, *Diplocodrus exsangui*; *i*, similar view of *Roeconota annulicornis*; *m*, similar view of *Conorhinus sanguisugus*; *n*, *Sinea diadema*; *o*, *Prionidus cristatus*; *p*, *Gelastocoris oculatus*; *q*, anaphase chromosomes of the same species; *r*, the XY-group, from the second division, *Acholla multispinosa*; *s*, diagram, slightly modified from Payne, to show the distribution of the XY-components in the second division of the same species.

latter being usually in close contact and in later anaphases sometimes hardly separable (5*g*), though now and then all three components are for a time strung separately along the spindle in the early anaphases, so that no doubt of their distinctness can exist (5*f*). Comparison of the diploid groups of the two sexes shows that those of the male contain but three of these small chromosomes and those of the female four, the total respective numbers being 27 and 28 (instead of 16 in both sexes, as in the A form).

These facts make it perfectly clear that one of the small chromosomes in the male passes to the male-producing pole, and therefore corresponds to the Y-chromosome; while the other two, *taken together*, represent the large idiochromosome, or X-chromosome, of the A form—precisely as in the reduvioids the single X-chromosome of *Diplocodus* is represented by a double element in *Fitchia*, *Rocconota* or *Conorhinus* (Payne). Had we no other evidence on this point we might assume simply that the original X-chromosome has divided into two equivalent X-chromosomes. But there are other facts that give reason for the conclusion that the breaking up of a single X-chromosome into separate components means something more than this. In the B form, as in *Fitchia* or *Rocconota* (fig. 5*l*), the X-element consists of two equal components, but in *Conorhinus* the two components are always of unequal size (5*m*). In *Prionidus* and in *Sinea* there are three equal components (5*n*, *o*), in *Gelastocoris* four equal ones (5*p*, and in *Acholla multispinosa* five, of which two are relatively large and equal and three very small (5*r*, *s*). In every case these components, though quite separate in the diploid groups (and usually also in the first spermatocyte-division) act as a unit in the second division, though not fused, and pass together to the female-producing pole (Payne, '09, '10).

In the foregoing examples the X-element is accompanied by a synaptic mate or Y-chromosome. The following are examples of a similar breaking up of the X-element into separate components when such a synaptic mate is missing. In *Phylloxera* (Morgan) the X-element consists of two unequal components, sometimes separate, sometimes fused together. In *Syromastes* (Gross,

Wilson) it consists of two unequal components, always separate, in the diploid groups but closely in contact (not fused) in both spermatocyte-divisions. The recent work of Guyer ('10) indicates a similar condition in the X-element of man. In *Agalena* (Wallace) there are two equal components, always separate. Finally, in *Ascaris lumbricoides* (Edwards, '10) there are five components, separate, and scattered in the diploid groups but closely associated in the spermatocyte-divisions.

In all these cases the significant fact is that not only the number but also the size-relations of these components are constant; and in many of these forms this fact may be seen in such multitudes of cells, and with such schematic clearness, as to leave no manner of doubt. It seems impossible to understand this series of phenomena unless we assume that the single X-chromosome is essentially a compound body—*i.e.*, one that consists of different constituents that tend to segregate out into separate chromosomes. We are led to suspect, further, that the composition of the X-element, even when it is a single chromosome, may differ widely in different species because of its great variations of size as between different species. For instance, in the family of Coreidae it is in some cases very large (*Protenor*), in others of middle size (*Chelinidea*, *Narnia*, *Anasa*), in others one of the smallest of the chromosomes (*Alydus*). Similar examples might be given from other groups.

In the case of *Thyanta*, therefore, it seems a fair assumption that the double X-element of the B form likewise represents at least a partial segregation of the X-chromatin from other constituents; and the latter may plausibly be regarded as representing the 'Y-chromatin' of the original X-member of the pair. In other words, we may think of the triad element as a YY-pair, one member of which is accompanied by a separate X-chromosome. In accordance with this its formula should be X.Y.Y, while that of the A form is XY.Y; and this may also be extended to other forms of similar type. If this be admissible, the male formula, as regards essential chromatin-content, becomes in general XY.Y and the female XY.XY, both sexes being homozygous for the Y-constituents, while in respect to X the male is heterozygous,

the female homozygous. The puzzle of the Y-chromosome would thus be solved; for although a separate Y-chromosome, when present, is confined to the male line, its disappearance only reduces the male from a homozygote to a heterozygote in respect to the Y-chromatin, and the introduction of supernumerary Y-chromosomes into the female (as in *Metapodius*) brings in no new element.



Fig. 6 Compound groups formed by union of the X-chromosome with other chromosomes in the Orthoptera. (*a* and *b*, from Sinéty, the others from McClung.) *a*, triad group; first division of *Leptynia*, metaphase; *b*, division of similar triad in *Dixippus*; *c*, triad group formed by union of the X-chromosome with one of the bivalents, first spermatocyte-prophase, *Hesperotettix*; *d*, the same element from a metaphase group; *e*, the same element in the ensuing interkinesis; *f*, the compound element of *Mermiria*, from a first spermatocyte prophase; *g*, the same element in the metaphase (now, according to McClung, united to a second bivalent to form a pentad); *h*, the same element after its division, in the ensuing telophase.

The same general view as that outlined above is suggested by the constant relation known to exist in some cases between the X-chromosome and a particular pair of the 'ordinary chromosomes.' The first observed case of this was recorded by Sinéty ('01) in the phasmid genera *Leptynia* and *Dixippus* (fig. 6 *a, b*), where the X-chromosome is always attached to one of the bivalents in the

first spermatocyte-division, and passes with one half of the bivalent to one pole. Since the spermatogonial number in *Leptynia* (36) is an even one and twice that of the separate chromosomes present in the first spermatocyte-division, it may be inferred that the X-element is already united with one of the ordinary chromosomes in the spermatogonia, though Sinéty does not state this. Somewhat later McClung ('05) discovered essentially similar relations in the grasshoppers *Hesperotettix* and *Anabrus* (fig. 6, *c-e*), and in case of the first named form was able to establish the important fact that it is always the same particular bivalent with which the X-chromosome is thus associated. In respect to the intimacy of this association, a progressive series seems to exist, since in *Leptynia* it seems to take place in the spermatogonia, in *Hesperotettix* only in the prophases of the first spermatocyte-division, while in *Thyanta* the union is only effected after the first division is completed.

Finally, the recent observations of Boring ('09), Boveri ('09) and Edwards ('10) seem to establish the fact that in *Ascaris megalocephala* the X-element, whether in the diploid groups or in the maturation-divisions, may either appear as a separate chromosome (which has the usual behavior of an accessory chromosome) or may be indistinguishably fused with one of the ordinary chromosomes.

These relations may, of course, be the result of a secondary coupling; and I myself formerly so interpreted them ('09*c*). But in view of what is seen in *Thyanta* or the reduvioids we may well keep in mind the possibility that they are expressions or remnants of a more primitive association, like that which I have assumed for an original XY-pair. Whatever be their origin, the effect is the same—a definite linking of the X-chromatin with that of one of the other pairs.

Fig. 7 shows, in purely schematic form, the general conception of these relations that has been suggested above, the X-chromatin being everywhere represented in black. A is the primitive XY-pair from which all the other types may have been derived. By simple reduction of such a pair arises the ordinary or typical idiochromosome-pair (B); and from either A or B may be derived

the other types (C-G),<sup>s</sup> or the more complicated ones shown in fig. 5. I represents the possible mode of separation of the X-element from a YY-pair, as suggested by Stevens; and this may be realized in *Ascaris megalocephala* (H). J and K are schemes of the relations seen in *Hesperotettix*, *Anabrus* and *Mermiria* (cf. fig. 6). These may be direct derivatives of a primitive XY-pair, as the diagram suggests, or may be a result

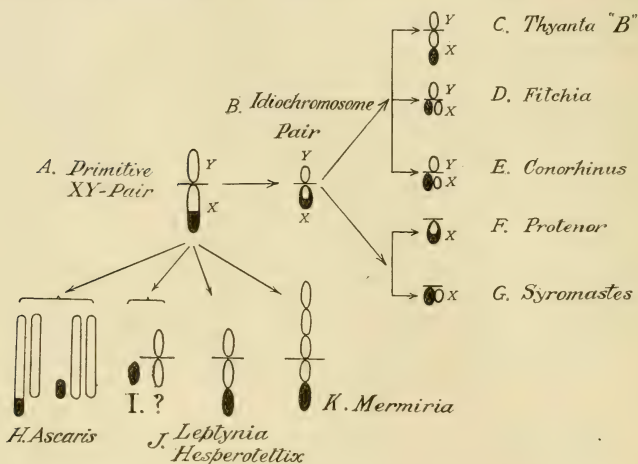


Fig. 7 Diagram illustrating the possible relation of the various types of idiochromosomes to a primitive XY-pair. Explanation in text.

of secondary coupling of X with other elements. In either case X may itself have such a composition as is indicated in F (*Protenor*).

(c) *Sex-limited heredity*. (1) The foregoing considerations have an important bearing on the problem of sex-limited heredity, for they give us a very definite view of how such heredity may be effected. It is not my intention to consider this subject in ex-

<sup>s</sup> These figures are not intended to indicate the precise mode of segregation of the X- and Y-chromatins of the X-element, but only illustrate possible modes.

*tenso*; but I wish to indicate some of the possibilities that have been opened by the cytological results, even at the risk of offering what may be regarded as too speculative a treatment of the matter. It is obvious that *any recessive mutation should exhibit sex-limited heredity when crossed with the normal or dominant form, if it be due to a factor contained in (or omitted from) the X-element*. For instance, in the remarkable *Drosophila* mutants discovered by Morgan ('10) the experimental data establish the fact that white eye-color (which seems to follow the same type of heredity as color-blindness in man) is linked with a sex-determining factor in such a way that when the white-eyed male is crossed with the normal red-eyed female, the former character is never transmitted from father to son, but through the daughters to some of the grandsons (theoretically to 50 per cent), though the daughters are not themselves white-eyed; that is, after such an initial cross, white eyes fail to appear in the  $F_1$  generation in either sex and in the  $F_2$  generation appear only in some of the males. As Morgan points out, this follows as a matter of course if the factor for white eye be identical with, or linked with, a sex-determining factor in respect to which the male is heterozygous or simplex, the female homozygous or duplex. The X-element exactly corresponds in mode of distribution to such a sex-determining factor; for this chromosome, too, is simplex in the male, duplex in the female and its introduction into the egg by the spermatozoon produces the female condition, its absence the male. This chromosome therefore, as I have shown ('06), is never transmitted from father to son, but always from father to daughter. Conversely, the male zygote always receives this chromosome from the mother. So precise is the correspondence of all this with the course of sex-limited heredity of this type that it is difficult to resist the conclusion that we have before us the actual mechanism of such heredity—in other words, that some factor essential for sex is associated in the X-element with one that is responsible for the sex-limited character.

This will be made clearer by the accompanying diagram (fig. 8) where the X-element assumed to be responsible for a recessive sex-limited character is underscored (X). This character may

be regarded as due to the absence of some particular constituent that is present in the normal X-element.

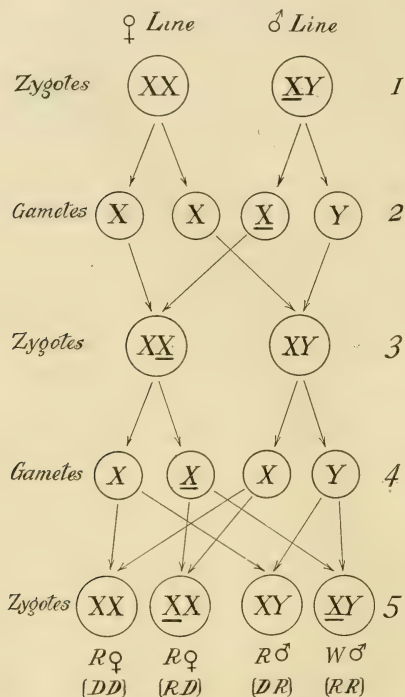


Fig. 8 Diagram of the distribution of the X- and Y-elements in successive generations, illustrating sex-limited heredity. The underscored X-element (X) is assumed to bear a factor for a recessive character (e.g., white eye-color), while X represents the normal or dominant character (e.g., red eye-color). Y (being the absence of X) likewise represents the recessive character.

Upon pairing the affected male (XY) with the normal female (XX) there are in the  $F_1$  generation but two possible combinations, XX and XY. The affected X-chromosome here passes

into the female, and the male is normal; but the female of course likewise shows only the normal (dominant) character. In the following  $F_2$  generation (5) there are four possible combinations  $XX$ ,  $\underline{XX}$ ,  $XY$  and  $\underline{XY}$ , two of each sex. Though  $\underline{X}$  is present in half of each sex, the character appears only in the males,  $\underline{XY}$ , again because of its recessive nature. By crossing together males of the composition  $\underline{XY}$  and females of composition  $\underline{XX}$ , some of the resulting females will have the composition  $\underline{XX}$ , and the sex-limited character is thus made to appear in the female.

When the female is the heterozygous or digametic sex—as in sea-urchins, in *Abraxas*, the Plymouth Rock fowls, etc.—exactly the converse assumption has to be made. Here, as Spillman ('08) and Castle ('09) have pointed out, the observed results follow if the sex-limited character (*e.g.*, lacticolor color-pattern in *Abraxas*) be allelomorphic to, or the synaptic mate of, a sex-determining factor,  $X$ , that is present as a single element in the female but absent in the male. The formulas now become<sup>9</sup> (as Spillman has indicated)  $XG$  ( $\varphi$  grossulariata),  $GG$  ( $\sigma$  gross.)  $X\underline{G}$  ( $\varphi$  lacticolor) and  $\underline{GG}$  ( $\sigma$  lact).  $XG \times GG$  then gives in  $F_1$   $XG$  and  $\underline{GG}$  (gross.  $\varphi$  and  $\sigma$ ),  $\underline{G}$  having passed from the female to the male. The following cross,  $XG \times \underline{GG}$  gives in  $F_2$  the four types  $XG$ ,  $X\underline{G}$ ,  $GG$  and  $\underline{GG}$ ,—*i.e.*, grossulariata appearing in both sexes but lacticolor only in the female. By crossing  $X\underline{G}$  with  $\underline{GG}$  some of the progeny will have the composition  $\underline{GG}$  ( $\sigma$  lacticolor). The other combinations follow as a matter of course.

This interpretation is in all respects the exact converse of that made in the case of *Drosophila*, which is also the case with

<sup>9</sup> These formulas are in substance the same as those stated by Mr. Spillman in a private letter to the writer, and are a simplified form of those suggested by Castle ('09). The interpretation thus given seems both the simplest and the most satisfactory from the cytological point of view of all those that have been offered. It obviates the cytological difficulties that I urged ('09) against Castle's formulas, and renders unnecessary the secondary couplings that I suggested. All these ways of formulating the matter conform, of course, to the same principle and differ only in details of statement. Whether the synaptic mate of  $X$  is directly comparable to the  $Y$ -chromosome of other insects (in which case the female formula becomes  $XY$  and the male  $YY$ ) is an open question.

the experimental results, as Morgan has pointed out. It seems probable that all the observed phenomena may be reduced in principle to one or the other of these schemes, though many modifications or complexities of detail probably exist. A possible basis for many such modifications seems to be provided by the cytological facts already known.

(2) We might assume that in cases of the first type (*e.g.*, *Drosophila*) both sex and the characters associated with it are determined by the same chromatin; and such a possibility should certainly be borne in mind. But in view of the widely different nature of the characters already known to exhibit sex-limited heredity it seems improbable that we can regard them as all alike due to the same chromatin. In the light of the conclusions that have been indicated in regard to the composition of the X-element, it seems more probable that such characters may be determined by the various other forms of chromatin ('Y-chromatin') associated with the X-chromatin. If these constituents be identical with those contained in the free Y-chromosome (the synaptic mate of X) sex-limited heredity would of course not appear, since the two members of the pair would be homozygous in this respect. It should make its appearance as a result of the dropping out, or other modification, of certain Y-constituents of the X-element, and such a mutation might arise in either sex.

We may perceive here the possibility of understanding many different kinds of sex-limited heredity, perhaps of complex types that have not yet been made known. Such a possibility is suggested, for example, by the remarkable relation discovered by McClung ('05) in *Mermiria* (fig. 6 *f-h*, fig. 7 in diagram), where the X-chromosome is in the first spermatocyte-division attached at one end to a linear chain of four other elements to form a pentad complex, to which may be given the formula  $XA.ABB$ . This so divides as to separate  $XA$  from  $ABB$ . The interpretation to be placed upon this is a puzzling question under any view, and apparently must await more extended studies on both sexes, perhaps also on other forms, before it can be fully cleared up. Even here the possibility exists, I think, that the entire complex may have arisen by the differentiation of a single original XY-pair;

but this question is clearly not yet ready for discussion. However such associations have arisen, the result is equally applicable to the explanation of sex-limited heredity.

(d) *Secondary sexual characters.* Castle ('09) has offered the interesting suggestion that the free Y-chromosome may be responsible for the determination of secondary sexual characters in the male. Though I have criticized this view ('09c) I now believe it may be true for certain cases. It is obviously excluded when the Y-chromosome is missing; and since nearly related species—in *Metapodius* even different individuals of the same species—show the same or similar secondary male characters whether this chromosome be present or absent, it seems probable that these characters are in general determined in some other way. But if, as I have suggested, sex-limited heredity may arise through a modification of the Y-constituents of the X-element, it follows that the YY-pair thereby becomes heterozygous. In such case, the free Y-chromosome, being confined to the male line, should continue to represent characters that are no longer present in the female, and hence would be indistinguishable from secondary male characters otherwise determined. It has further become evident (as is indicated below) that the chromosome-groups are so plastic that their specific composition may vary widely from species to species. It may very well be, therefore, that Castle's suggestion may apply to some forms.

#### 6. *Modes in which the chromosome-number may change*

The constant and characteristic duality of the 'd-chromosome' in the second division suggests a series of questions regarding the mode in which the chromosome-number may change that have an important bearing on those already considered. The appearance of this chromosome must suggest to any observer that it is a compound body, consisting of two closely united components that are invariably associated in a definite way; but it is especially noteworthy that its duality does not certainly appear before the last division. This case must be added to the steadily increasing evidence that chromosomes which appear single and homoge-

neous to the eye may nevertheless be compound bodies. An important part of it is derived from the modifications of the X-element reviewed above; but the evidence is now being extended to the 'autosomes' or ordinary chromosomes as well. The double chromosome of *Nezara* suggests either the initial stages of a separation of one chromosome into two or the reverse process—in either case that we have before us one way in which the number, and the composition, of the chromosomes may change from species to species. This is supported by the recent observations of Miss E. N. Browne ('10) on *Notonecta*. In *N. undulata* there are always, in addition to a typical unequal XY pair, two small chromosomes that appear in all the divisions as separate elements. In *N. irrorata* there is always but one such chromosome, the total number in each division being accordingly one less than in *N. irrorata*. *N. insulata* presents a condition exactly intermediate, there being sometimes one and sometimes two such small chromosomes. When, however, but one seems to be present, the second may often be seen closely adherent to one of the larger chromosomes; and the latter may positively be identified, by its size, as *always the same one*. It can hardly be doubted, as the author points out, that we here see three stages in a process by which the chromosome-number is changing, either by the fusion of two originally separate chromosomes, or by the separation of one into two. It is of the utmost importance that this process affects a chromosome that can be positively identified as the same in each case; for this proves that the change is not an indefinite fluctuation but the expression of a perfectly orderly process. While there is here (as in the case of the *d*-chromosome of *Nezara*) no way of knowing in which direction the change is taking place, either alternative involves the conception that the individual chromosomes may be compound bodies, whether as a result of previous fusion or as possible starting points for a subsequent segregation.

The facts now known indicate at least four possible ways in which the chromosome-number (and in three of these also the composition of the individual chromosomes, may change from species to species.

One is that suggested by the foregoing phenomena, *i.e.*, the gradual fusion of separate chromosomes into one or the reverse process.

A second mode may be the gradual reduction and final disappearance of particular chromosome-pairs, as was suggested by Paulmier ('99), and afterwards by Montgomery and myself, in case of the microchromosomes, or '*m*-chromosomes' of the co-reid Hemiptera. In respect to the size of these chromosomes, a graded series may be traced from forms in which they are very large (as in *Protenor*) through those where they are of intermediate size down to cases where they are very small (as in *Archimerus*) and finally to such a condition as that seen in *Pachylis* (fig. 9 *j-l*) where they are almost as minute as centrioles and may almost be regarded as vestigial. Four of these stages are shown in fig. 9. In *Protenor* (*a-c*) the *m*-chromosomes are so nearly of the same size as the next smallest pair that they often can not be positively identified in the spermatogonial groups. In *Leptoglossus phyllopus* (*d-f*) they are always recognizable, though not much smaller than the next pair. In *L. oppositus* or *L. corculus* they are a little smaller. In *Anasa* (the form in which they were first discovered by Paulmier) they are of middle size (*g-i*), representing perhaps a fair average of the group. Several other genera (*e.g.*, *Metapodius*) show intermediate stages between this condition and that seen in *Archimerus* (figured in my second 'Study,' and more recently by Morrill) where the *m*-chromosomes are almost as small as in *Pachylis*. It is most remarkable that throughout this whole series the *m*-chromosomes exhibit essentially the same behavior (Wilson, '05b, '06), usually remaining separate throughout the entire growth-period and only conjugating in the final prophase of the first spermatocyte-division, to form a bivalent which with rare exceptions occupies the center of the metaphase group; in some forms, also (*e.g.*, *Protenor*, *Alydus*) they show a marked tendency to condense at a much earlier period than the other chromosomes. The *m*-chromosomes of *Pachylis*, excessively minute though they are, exhibit a behavior in all respects as constant and characteristic as even the largest of the series. In the *Lygaeidae* they seem to be present in some

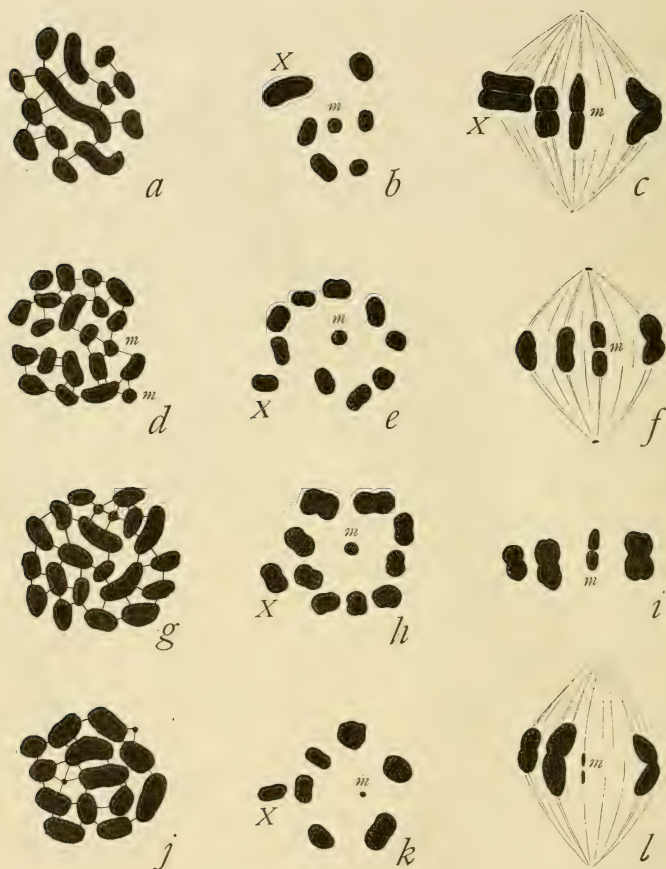


Fig. 9 Comparison of the *m*-chromosomes in Hemiptera. (In each horizontal row are shown at the left a spermatogonial group, in the middle a polar view of the first spermatocyte-division, at the right a side-view of the same division.) *a-c*, *Protenor belfragei*; *d-f*, *Leptoglossus phyllopus*; *g-i*, *Anasa tristis*; *j-l*, *Pachylis gigas*.

species (*Oedancala*, *t. Montgomery*), in others absent (*Lygaeus*). In the *Pyrrhocoridae* (*Pyrrhocoris*, *Largus*) they are absent as far as known. So characteristic is the behavior of these chromosomes as to leave not the least doubt of their essential identity throughout the whole series; and this series may be regarded as a progressive one, in one direction or the other, with the same reason as in case of any other graded series of morphological characters. The series thus shown in case of the *m*-chromosomes is as gradual and complete as in case of the Y-chromosome, and may with the same degree of probability be regarded as a descending one.

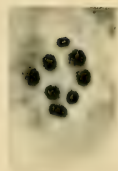
Thirdly, it is probable that the chromosome-number may change by sudden mutations that produce extensive redistributions of the chromatin-substance without involving any commensurate change in its essential content. Were gradual changes, chromosome by chromosome, the usual mode of modification, we should certainly expect to find such conditions as are seen in *Nezara*, in *Notonecta*, or in the *Coreidae*, more frequently. In some groups, however, we find wide differences of chromosome-number between species even of the same genus, and even between those that are very nearly related, without any accompanying evidence of a gradual process of transition—for instance, among the aphids and phylloxerans (Stevens, Morgan) or in the heteropterous genera *Banasa* and *Thyanta*. (Wilson, '09*d.*) In *Banasa dimidiata* the diploid number is 16 in both sexes, in the nearly related *B. calva* 26. Of the two races of *Thyanta* *custator* described above, apparently identical in other visible characters, one has in both sexes the diploid number 16, with a simple X-chromosome, while in the other the diploid number of the male is 27 and that of the female 28, and the X-chromosome consists of two components. It is improbable that the differentiation of these two forms has been accomplished by gradual modifications, chromosome by chromosome. It seems far more likely that the change took place by sudden mutation, involving a redistribution of the nuclear material which changed its form but not in an appreciable degree its substance. In the well known case of *Oenothera gigas*, derived by sudden mutation from *Oe. Lamareckiana*, a change by sudden mutation is known to be

## PLATE 1

## EXPLANATION OF FIGURES

All the figures from photographs of sections. Enlargement 1500 diameters.

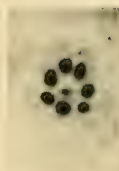
- 10, 11 First spermatocyte-division (N. hiliaris)
- 12, 13 The same (N. viridula)
- 14, 15 Second spermatocyte-division (hiliaris)
- 16-25 Side views of second division (hiliaris). The XY-pair shown in 16-23, the *d*-chromosome in 16, 17, 20, 24, 25; the small chromosome is evident in 10, 12, 13, 14, 15, 17, 18.
- 22 Initial separation of X and Y
- 23 Early anaphase, X and Y separating near the center (hiliaris)
- 26-28 Nuclei from the growth-period, showing chromosome-nucleolus and plasmosome (hiliaris)
- 29 Corresponding stage (viridula)



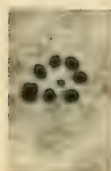
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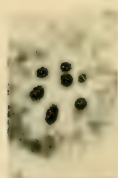
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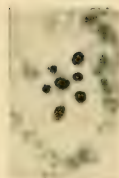
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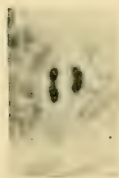
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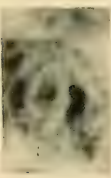
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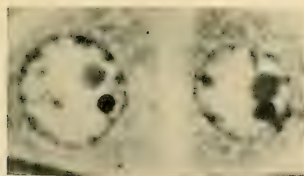
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a fact (Lutz, '07; Gates, '08), though it may be due in this instance to a simple doubling of the whole group. Such cases led me several years ago to the conclusion "that the nucleus consists of many different materials that segregate in a particular pattern . . . and that the particular form of segregation may readily change from species to species" (Wilson, '09*d*, p. 2).

Such changes must involve corresponding ones in the morphological and physiological value of the individual chromosomes; and we must accordingly recognize the probability that these individual values, though constant for the species, may change from species to species as readily as does the number. Despite the conformity to a general type often exhibited by particular genera or even by higher groups, the individual chromosomes are therefore *per se* of subordinate significance; and it may often be practically impossible to establish exact homologies between those of different species. How closely this bears on the origin of the diverse conditions seen in the composition of the XY-pair is obvious.

Lastly, it is almost certain that changes of number may sometimes arise as a result of abnormalities in the process of karyokinesis, such as the passage of both daughter-chromosomes, or of both members of a bivalent, to one pole. In *Metapodius* I found ('09*b*) direct evidence of this in case of the XY-pair itself, and endeavored to trace to this initial cause the remarkable variations of number that occur in this genus. Many other observers have recorded anomalies of this kind, in both plants and animals. It seems entirely possible, as has been suggested by McClung ('05) and by Gates('08) that definite mutations may be traceable to this cause; though probably such abnormalities may in general be expected to lead to pathological conditions.

#### CONCLUSION

Some of the suggestions offered in the foregoing discussion are admittedly of a somewhat speculative character; but they are not, as I venture to think, mere *a priori* constructions, but are forced upon our attention by the observed facts. The time has come

when we must take into account more fully than has yet been done the new complexities and possibilities that have continually been unfolded as we have made better acquaintance with the chromosomes. In this respect the advance of cytology has quite kept pace with that of the experimental study of heredity; and it has established so close and detailed a parallelism between the two orders of phenomena with which these studies are respectively engaged as to compel our closest attention.

Studies on the chromosomes have steadily accumulated evidence that in the distribution of these bodies we see a mechanism that *may* be competent to explain some of the most complicated of the phenomena that are being brought to light by the study of heredity. New and direct evidence that the chromosomes are in fact concerned with determination has been produced by recent experimental studies, notably by those of Herbst ('09) and Baltzer ('10) on hybrid sea-urchin eggs. But the interest of the chromosomes for the study of heredity is not lessened, as some writers have seemed to imply, if we take the view—it is in one sense almost self-evident—that they are not the exclusive factors of determination. Through their study we may gain an insight into the operation of heredity that is none the less real if the chromosomes be no more than one necessary link in a complicated chain of factors. From any point of view it is indeed remarkable that so complex a series of phenomena as is displayed, for example, in sex-limited heredity can be shown to run parallel to the distribution of definite structural elements, whose combinations and recombinations can in some measure actually be followed with the microscope. Until a better explanation of this parallelism is forthcoming we may be allowed to hold fast to the hypothesis, directly supported by so many other data, that it is due to a direct causal relation between these structural elements and the process of development.

A second point that may be emphasized is the remarkable constancy of the chromosome-relations in the species, and their no less remarkable plasticity in the higher groups. The scepticism that has been expressed in regard to constancy in the species finds, I think, no real justification in the facts. It is perfectly true that

individual fluctuations occasionally are seen in the number of the chromosomes, in the process of synapsis, in the distribution of the daughter-chromosomes, and in all other cytological phenomena. It is, however, also true that most observers who have made prolonged, detailed and comparative studies of any particular group, have sooner or later reached the conviction that in each species all the essential relations in the distribution of the chromosomes conform with wonderful fidelity to the specific type. So true is this that the species may often at once be identified by an experienced observer from a single chromosome-group at any stage of the maturation-process. No one, I believe, who has engaged for a series of years in the detailed study of such a group, for instance, as the Hemiptera or the Orthoptera, returning again and again to the scrutiny of the same material, can be shaken in the conviction that the distribution of the chromosomes follows a perfectly definite order, even though disturbances of that order now and then occur. But it is equally important to recognize the fact that this order has undergone many definite modifications of detail from species to species, and that while all cases exhibit certain fundamental common features, we cannot without actual observation predict the particular conditions in any given case. It is now evident that the larger groups vary materially in respect to specific conditions. For instance, in the orthopteran family of Acrididae (McClung) the relations seem to be far more uniform than such a group as the Hemiptera, where great specific diversity is exhibited, the details often changing from species to species in a surprising manner—witness the species of Aphis or Phylloxera (Stevens, Morgan), those of Acholla (Payne) or of Thyanta (Wilson). In these respects, too, the cytologist finds his experience running parallel to that of the experimenter on heredity; and here, once more, we find it difficult not to believe that both are studying, from different sides, essentially the same problem.

December 13, 1910.

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## THE TRANSPLANTATION OF OVARIES IN CHICKENS<sup>1</sup>

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Dr. C. C. Guthrie ('08) has reported the results of transplanting ovaries from black to white hens and vice versa. A black-plumaged hen furnished by transplantation with 'white' eggs and mated to a white cock gave "about equal numbers of white and spotted" chicks. Guthrie thinks that these black spots indicate that the black-plumaged foster-mother infected the engrafted 'white' eggs. So far Guthrie. But a person familiar with the results of hybridizing will appreciate that Guthrie's result is better explained on the assumption that the engrafted ovary was absorbed and that the white sperm fertilized the regenerated 'black' eggs of the black hen. For the white by black cross gives white offspring with black spots in the female chicks only, *i.e.*, half of all, as Guthrie found.

In a second set of experiments, Guthrie found that when a white hen carrying a 'black' ovary was mated to a White Leghorn male, the offspring were either white or black or spotted. Guthrie says: "The black, therefore, must have come through the black ovary." But the student of hybridization on poultry will recognize at once that, if the white-plumaged cock produced only 'white' germ cells, none of his offspring would be black even if the eggs were 'black.' Hence, the cock must have had 'black' germ cells and, very likely, the hen also, since 'White Leghorn' hens that carry 'black' germ cells are very common and frequently show, in adult life, a pure white plumage.

If two 'White Leghorns' with 'black' germ cells be mated expectation is that in four chicks one shall be black; one spotted, and

<sup>1</sup> A preliminary paper covering these results was read before the Society for Experimental Biology and Medicine, June 1910.

two white; Guthrie got five chicks, one black, one spotted and three white.

Guthrie found that a black hen containing a 'white' ovary, mated with a black cock gave black-plumaged chicks, of which two out of six had white feet. He concludes that the white condition of the feet must have come from the engrafted eggs of the White Leghorn. In criticism it must be pointed out that the cross, white egg  $\times$  black sperm, normally gives offspring whose plumage color is white, either pure or with black specks. The fact that all the offspring had black plumage proves that the eggs were the normal 'black' eggs regenerated by the black hen. The white toes are frequently found in the offspring of two black birds. Thus in my pen 1041 two extracted blacks (Sumatras) mated give ten black chicks in six of which white toes are recorded. The results of this cross of Guthrie's confirm the conclusion that the transplanted ovaries were not functional and that the normal ovaries had regenerated.

To test the possibility of such regeneration of ovaries I removed the ovaries of some hens in the autumn of 1909 and transplanted into them eggs from dissimilar hens. The operated birds were then mated to cocks resembling the soma of the so-called 'foster-mother.' Were there regeneration of the ovary the offspring should be of the straight breed; but if the 'grafts' persisted and became functional the chicks should be hybrids.

*Experiments 1 and 2, operations:* The protocol of the grafting operations is as follows:

No. 11379, pure-bred Dark Brahma bantam, hatched February, 1909; made to fast two days. On September 29, 1909, injected with 0.005 grain of atropin in 1 cc. of water, etherized in about twelve minutes and opened up between two left intercostals. Large ovary, badly torn in removal, removal tolerably complete. One piece of ovary from no. 11605 fastened by cotton thread to mesentery near attachment of ovary. Sewed up.

No. 11605, hatched March, 1909, from White Leghorn, -Houdan ancestry. Clean-footed, with five toes on each foot, V-comb, modified high nostril, plumage color white (with black recessive). On September 29, 1909, injected 0.005 grain atropin in 1 cc. of

water. Etherized in twenty minutes. Plucked feathers and opened body wall between last two ribs. Large ovary completely removed or nearly so, in three or four pieces. Hemorrhage slight. Stitched in small piece of ovary of no. 11379 to peritoneum near attachment of old ovary. Sewed up. Bird recovered rapidly. Some Dark Brahma in ancestry, but its characters had become eliminated.

RESULTS, *Experiment 1*. Mated in pen 1027, no. 11605 ♀ (with engrafted ovary from no. 11379, Dark Brahma) and 11291 ♂, straight Dark Brahma. Table 1 gives the juvenile characteristics of 1, the male; 2, the White Leghorn-Houdan, so-called foster-mother; 3, the hen from which the ovaries were transplanted; 4, expectation on the hypothesis that the graft succeeded; 5, expectation on the hypothesis that the graft failed and the proper ovary was regenerated; and 6, the observed characteristics of the young offspring.

An examination of table 1 shows at once that it cannot be true that the engrafted ovary replaced the hen's proper ovary, for if it had, columns six and four should agree. On the contrary, column six agrees essentially with column five and supports the hypothesis that the engrafted eggs did not become functional.

One discordant fact there is, however, namely, the occurrence in column six of three cases of cinnamon offspring. Such offspring are to be expected on the hypothesis that some eggs of the graft became functional. If that hypothesis be true, then the other characters of the same individuals should be like those of the pure Dark Brahma. Of the three the first has extra toes, split comb and a boot of one row; it is no Dark Brahma; the second has extra toes, wide nostril and a two rowed boot; it is not a Dark Brahma; and the third has really black down with some red at the tips, five toes on the right foot, a split comb and one row of feathers on the shank; so it is not a Dark Brahma. These therefore, are not from the engrafted Dark Brahma eggs. They represent cases of imperfect dominance of the black down over cinnamon. The conclusion to be drawn from this experiment is that the engrafted eggs did not mature in the foster-mother.

TABLE 1

Showing the results of mating an operated White Leghorn-Houdan hen with normal Dark Brahma cock. Characters in brackets are recessive.

CHARACTERISTICS	1. FATHER	2. "FOSTER-MOTHER"	3. SOURCE OF OVARY	4. EXPECTATION		6. REALIZATION 42 OFFSPRING
				1 X 3	5. 1 X 2	
Plumage color, juvenile.....	cinnamon; gray below	white (+ smoky) [black back]	light cinnamon; gray below	cinnamon; light below	50 per cent white or white + smoky; 50 per cent black back	white; white + specks (or smoked) 21 black, white below 18 } 21 cinnamon above, white below 3 }
Booting.....	heavy, 5 to 7 rows	absent	heavy, 5 to 7 rows	heavy	intermediate,	3 rows, 12; 2 rows, 15; 1 row, 1; 0 row, 1; unrecorded, 3
Comb.....	pea	Y [V, I]	pea	pea	3 to 0 rows pea, 50 per cent; split pea, 50 per cent	pea, 16; split, 23; unrecorded, 3
Number of toes to foot.....	4	5 [4]	4	4	4, 50 per cent 5, 50 per cent	4 toes, 18; 5 toes, 24
Nostril.....	narrow, grade 2	wide, grade 7	narrow, gr. 2	narrow, gr. 2	narrow, 50 per cent Interm. and wide, 50 per cent	narrow, 12; intermediate and wide, 12; unrecorded, 18

*Experiment No. 2.* No. 11379, Dark Brahma with engrafted ovary from no. 11605 (White Leghorn-Houdan) was mated in pen 1050 with 14122 $\sigma$ , a single-comb Black Minorca. Table 2 gives the juvenile characters of 1, the male parent; 2, the Dark Brahma, so-called foster-mother; 3, the hen from which the ovaries were transplanted; 4, the expectation of offspring on the assumption that the graft succeeded; and 5, that the graft failed and the proper ovary was regenerated; also, 6, the observed characters in the offspring.

Without exception the characters of the offspring are clearly those of the Dark Brahma  $\times$  Minorca cross and none of the White Leghorn or Houdan differential characters enter into their composition. The grafted ovary produced no eggs that developed, the extirpated ovary was regenerated.

*Experiments 3 and 4, operations.* The protocol of the grafting operations is as follows:

No. 11541 $\varphi$  is a white-plumaged hen derived from a cross between 8681 $\varphi$ , a White Leghorn-Minorca-Polish bird, and 7811 $\sigma$ , a Houdan cross hatched (in pen 905) in February, 1909; fasted two days. On October 2, 1909, injected with 0.005 grain atropin in 1 cc. of water; etherized and opened. Ovary very large, two large pieces (60 per cent) of ovary removed. Strong hemorrhage. Two small pieces of ovary from no. 11383 $\varphi$ , Dark Brahma, sewed with peritoneum close to ovarian artery. Sewed up. Bird slow in recovery.

No. 11383 $\varphi$ , straight Dark Brahma, hatched February, 1909, from mating 907: 7549. On October 2, 1909, injected with atropin, etherized and opened, ovaries small, incompletely removed. Two large pieces of ovary of no. 11541 sewed into peritoneum. Sewed up. Bird recovered rapidly.

RESULTS, *experiment 3.* No. 11541, White Leghorn-Black Minorca-Polish-Houdan hybrid, with engrafted ovary from No. 11383, Dark Brahma, was mated in pen 1027 with 11291 $\sigma$ , a pure bred Dark Brahma. The results of this mating are given in table 3.

*Experiment 4.* No. 11383 $\varphi$ , pure-bred Dark Brahma with engrafted ovary from no. 11541 (White Leghorn-Black Minorca-

TABLE 2

*Showing the results of mating an engrafted Dark Brahma hen with a normal single-comb Black Minorca cock. Characters in brackets are recessive.*

CHARACTERISTICS	1. FATHER	2. "FOSTER-MOTHER"	3. SOURCE OF OVARY	4. EXPECTATION		5.	6.	REALIZATION 8 OFFSPRING
Plumage color, juvenile.....	black; white below	cinnamon; gray below	white, or white with black back	1 × 3	1 × 2			
Beetling.....	absent	heavy, 5 to 7 rows	absent	absent	black; white below		All black with white below	
Comb.....	single, high	pea, low	Y [4, V]	I, 50 per cent Y, 50 per cent	present but light, 3 to 1 row pea, modified		2 rows, 6; 1 row, 2 8 pea	
Number of toes to foot.....	4	4	5 [4]	4, 50 per cent 5, 50 per cent	4		all 4	
Nostril.....	narrow	narrow, grade 2	wide, grade 7	intermediate	narrow		all narrow, grade 1 or 2	

TABLE 3

Showing the results of mating of Experiment 3. The juvenile characters are given of (1) the male parent; (2) the white, 'foster-mother'; (3) the hen from which the ovaries were borrowed; (4) the expectation in the offspring on the assumption that the graft succeeded; and, (5) that the graft failed and the proper ovary was regenerated; also (6) the observed characters in the offspring.

CHARACTERISTICS	1. FATHER	2. "FOSTER-MOTHER"	3. SOURCE OF OVARY	4. EXPECTATION		6. REALIZATION 9 OFFSPRING
				1 × 3	5. 1 × 2	
Plumage color, juvenile.....	cinnamon; gray below	white	cinnamon; gray below	cinnamon; gray below	white, or white specked	white, 6; white with specks, 3
Booting.....	heavy, 5 to 7 rows	absent	heavy, 5 to 7 rows	heavy, 5 to 7 rows	intermediate, 0 to 3 rows	2 rows, 2; 1 row, 3; 0 rows, 3.
Comb.....	Pea	V	pea	pea	split pea	pea, split 7; pea, split (?) 2
Number of toes to foot .....	4	4 [5]	4	4	4 over 50 per cent 5 less than 50 per cent	4, 7, 5, 2,
Nostril .....	narrow, grade 1 or 2	wide, grade 8	narrow, grade 1 or 2	narrow, grade 1 or 2	narrow, 50 per cent intermediate and wide 50 per cent	grade 3, 2; grade 2, 3; grade, 1, 4
Erectness of crest feathers.....	absent	present	absent	absent	present	present, 2; unrecorded, 7 <sup>1</sup>

<sup>1</sup> No record was obtained from the chicks that died early, as erectness does not show until some time after hatching.

Polish—Houdan hybrid) was mated with a Black Minorca 14122 ♂. The results of this mating are given in table 4.

*Experiment 5.* No. 11693 ♀, used in this experiment, is a white bird that had 'smoke' on down when hatched. It is of somewhat complex origin. Its mother was an F<sub>1</sub> hybrid between a Black Spanish cock and a White Leghorn; its father had the same elements and also white Silkie in its ancestry. No. 11693 has, consequently, black recessive. It has a single comb, is free of the skin pigment of the Silkie, is clean-shanked and has four toes on the right foot and five on the left.

On September 19, 1909, this pullet (which was hatched March, 1910) was treated with atropin, etherized during half an hour and opened as usual between the last two ribs. All of the ovary, as far as could be seen, was removed. Pieces of ovary from no. 11280 ♀ (a straight-bred Dark Brahma bantam) were placed in contact with the peritoneum, near the removed ovary, but not stitched in, as the bird showed signs of succumbing. The cut was sewed up and the bird set aside where it lay quiet for half an hour.<sup>2</sup> The Dark Brahma from which the ovary (whose eggs measured 0.5 mm. in diameter) was removed died in consequences of hemorrhage.

Later No. 11693 was mated with 11291 ♂ (in mating 1027: 11693). He is a straight-bred dark Brahma bantam cock, used also in experiments 1 and 3. The results are shown in table 5.

*Experiment 6.* No. 11826 ♀, hatched March, 1909, a pure bred Dark Brahma was opened October 2, 1909, and ovary imperfectly removed. Ovary of no. 12550 (a White Leghorn-Minorca-Polish-Houdan hybrid) sewed on to peritoneum at point of removal. The ovary had been kept out of body of hen about ten minutes, but covered and moist.

In the late winter of 1910 no. 11826 ♀ was mated in pen 1050 with 14122 ♂, a single-comb Black Minorca. The results are given in table 6.

<sup>2</sup> See postscript.

TABLE 4

Showing the results of Experiment 4. The juvenile characters are given of (1) the male parent; (2) the Dark Brahma 'foster-mother'; (3) the hen from which the ovaries were borrowed; (4) the expectation in the offspring on the assumption that the graft succeeded; and, (5) that it failed and the proper ovary was regenerated; also (6) the observed characters in the offspring.

CHARACTERISTICS	1. FATHER	2. "FOSTER MOTHER"	3. SOURCE OF OVARY	4. EXPECTATION		6. REALIZATION 14 OFFSPRING
				1 X 3	5. 1 X 2	
Plumage color, juvenile.....	black; white below	cinnamon; gray below	white [black]	white 50 per cent; black 50 per cent;	black; white below	black, white below, 14
Beaking.....	absent	heavy, 5 to 7 rows	absent	absent	present, but light,	3 rows, 4; 1 row, 8; 0 row, 2
Comb.....	single	pea	V	Y-comb	0 to 3 rows pea, modified	pea, 13; uncertain, pea or single, 1
Number of toes to foot.....	4	4	4 [5]	4, over 50 per cent 5, less than 50 per cent	all 4	4, 14
Nostril.....	narrow, grade 1	narrow, grade 2 or 1	high, grade 9	intermediate	narrow	grade 2 or 1, all
Erectness of crest feathers.....	absent	absent	present	present	absent	absent in all

TABLE 5

Results of mating No. 11693 ♀ (with engrafted Dark Brahma ovary) and No. 11291 ♂, a pure-bred Dark Brahma. The juvenile characters are given (1) of the father; (2) of the white so-called 'foster-mother'; (3) of the Dark Brahma hen from which the ovaries were transplanted; (4) the expectation in the offspring on the assumption that the graft succeeded; and (5) that it failed; also (6) the observed characters in the offspring.

The low proportion of whites in the offspring is remarkable. It is no evidence of persistence of the engrafted tissue. The single case of a chick with cinnamon back is doubtless to be ascribed to imperfection of dominance.

CHARACTERISTICS	1. FATHER	2. "FOSTER-MOTHER"	3. SOURCE OF OVARY	4. EXPECTATION		6. REALIZATION 18 OFFSPRING
				1 × 3	5. 1 × 2	
Plumage color, juvenile.....	cinnamon back; gray belly	white [black]	cinnamon back; gray below	cinnamon back; gray below	white, and white spotted, 50 per cent; black, 50 per cent	white, black spotted, 2; black (white belly), 15; cinnamon back, 1
Booting.....	heavy, 5 to 7 rows	absent	heavy, 5 to 7 rows	heavy, 5 to 7 rows	slight, 0 to 3 rows	3 rows, 2; 2 rows, 9; 1 row, 3; unre- corded, 4
Comb.....	pea	single	pea	pea	pea, modified	pea, usually high, 11; unrecorded, 7
Number of toes to foot.....	4	4 right; 5 left	4	4	4, over 50 per cent 5, less than 50 per cent	4 toes, 15; 5 toes, 3

TABLE 6

Showing the results of the mating, Experiment 6. The juvenile characters are given of (1) the male (Black Minorca) parent; (2) the Dark Brahma 'foster-mother'; (3) the hen from which the ovaries were removed; (4) the expectation in the offspring on the assumption that the graft succeeded, and (5) that the graft failed; also (6) the observed characters in the offspring.

CHARACTERISTICS	1. FATHER	2. "FOSTER-MOTHER"	3. SOURCE OF OVARY	4. EXPECTATION	5.	6. REALIZATION
				1 X 3	1 X 2	
Plumage color, juvenile.....	black; white below	cinnamon above; gray below	white [black and white]	white, 25 per cent; white, black specks, 25 per cent; black and white 50 per cent	All black; white below	black, white below, 19
Booting.....	absent	heavy, 5 to 7 rows	absent	absent	intermediate, 0 to 3 rows	2 rows, 4; unrecorded, 1 3 rows, 13 4 rows, 1
Comb.....	single	pea	V	Y	pea, modified	pea, 18 unrecorded 1
Nostril.....	narrow, grade 1	low, grade 1 or 2	wide, grade 8	Intermediate grade 5 to 2	low, grade 1 or 2	low, grade 1 or 2, 15; unrecorded, 4

## CONCLUSIONS

In the six experiments described above there is no evidence that the engrafted ovary ever became functional but all results are in accord with the conclusion that the more or less completely extirpated ovary regenerated and produced an abundance of eggs. With the results the data of Dr. Guthrie's paper are not in discord. His data, like ours, furnish no evidence for the survival of the engrafted ovaries, far less of an effect of the soma of the foster-mother on the introduced germ plasm.

Cold Spring Harbor, N. Y.  
September 26, 1910.

## POSTSCRIPT

On January 4, 1911, No. 11693 ♀ was killed and opened on the left side. An ovary of fairly typical size for a hen entering her second year of laying was found. It contained numerous eggs, 4 to 5 mm. in diameter. Slightly ventrad of the main artery of the ovary is an irregular mass  $5 \times 4 \times 2$  mm. of cheesy consistency, imbedded in and covered by peritoneum. Its general appearance is that of a dried, hardened ovary, with clear traces of follicles. It doubtless represents the engrafted ovary, entirely encysted in the peritoneum.

January 30, 1911.

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# THE EFFECTS OF INBREEDING AND SELECTION ON THE FERTILITY, VIGOR AND SEX RATIO OF DROSOPHILA AMPELOPHILA

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## CONTENTS

Introductory.....	124
Material and methods .....	124
Inbreeding and selection on fertility and vigor.....	126
1 Introductory.....	126
2 Sterility.....	127
<i>a</i> Character of the sterility .....	127
<i>b</i> Degrees of sterility.....	128
3 Inbreeding and vigor.....	131
4 Sterility and selection.....	134
5 Discussion of results.....	138
Sex-ratio and selection.....	141
1 Introductory.....	141
2 The normal sex-ratio.....	141
3 Control of sex-ratio by selection.....	142
<i>a</i> History of strain 206.....	143
<i>b</i> History of strain 207.....	147
<i>c</i> Discussion.....	147
4 Influence of male and female in determining the sex-ratio.....	148
5 Discussion of results on sex-ratio .....	151
Summary.....	153
Literature cited.....	154

## INTRODUCTORY

The present report includes the results of two series of experiments on the fruit fly—*Drosophila ampelophila*. One set concerns itself primarily with the effects of inbreeding and the other with sex-ratios. The experiments on inbreeding grew out of work I had been carrying on on hybridization. In these hybridization experiments the effects on the developmental processes of hybrids between species too remotely related were especially emphasized. The converse of these experiments was, naturally, to study the effect upon the young between individuals too closely related. Fishes, upon which all my experiments in hybridization were made, do not lend themselves for purposes of inbreeding without elaborate breeding facilities. Mice seemed suitable for this purpose but, both at the outset of these experiments and since, these creatures have proven miserable failures in my hands. Among the insects, I tried the common willow beetle but this proved to throw only one generation annually in this latitude. It was desirable to have an animal with a brief life history, whose food could be easily obtained at all seasons and in which the sexes could be readily distinguished. In these respects the fruit fly is almost ideal. The facts herein considered confine themselves to this species.

The experiments on sex-ratio suggested themselves in connection with the inbreeding experiments and so were carried out along with the latter and after they were completed.

## MATERIAL AND METHODS

The strain which is mostly under discussion in my inbreeding experiments came from a well-filled female that was taken from the window of my residence in Bloomington. Other strains were started at the onset. Some of these came from the banana bunches at the various groceries and others came from fruit which I had laid out for this purpose. None of these were carried further than two or three generations excepting two, called 6 and 7 in my records. The latter was discontinued after the tenth generation

since it had been from the beginning apparently less prolific. The strain 6 was carried for over seventy-five generations and is the one on which the experiments in inbreeding of this report are based.

For vivaria, tall stender dishes, tumblers, quinine bottles and lamp chimneys were given a trial. They were discarded in favor of 8-dram shell vials. These were compact, so that a large number of matings could be kept in a small space, and they were most convenient in manipulating the pairs during the frequent changes to new cages that was necessary all along. The open end of the shell vial was closed with a plug of absorbent cotton, not too compact, so as to afford some ventilation. The flies are strongly positive to light, so that the vials could be laid with their bottom toward the light and the cotton plug removed with safety for the introduction of food etc. Small trays holding fifteen of these vials were used and in this way the experiments could be readily and compactly stored in the incubator, or they could be packed into a valise to be taken along wherever I went. The food was exclusively well-ripened bananas. To prevent the larvae from pupating in the food, narrow strips of blotter or filter paper were introduced in which they seemed to be especially fond of pupating. It is, of course, apparent that the greatest care had to be taken to avoid contamination from flies without. The stock food had to be scrupulously watched and the instruments kept clean to avoid the introduction of eggs laid on them by extraneous females. The bananas, especially, as they come from the stores, are likely to be infected with eggs and larvae if the skin be in any way bruised or split.

The brothers and sisters were paired off, always within the first ten or twelve hours of their life as imagoes. Up to this time mating has not occurred. In fact I have never found a pair that copulated during the first twenty-four hours or, if so, that produced fertile eggs.

## INBREEDING AND SELECTION ON FERTILITY AND VIGOR

*1. Introductory*

That continued inbreeding acts deleteriously on the fertility and vitality of a race is a belief so firmly and generally established that it is seldom questioned. This has its origin largely in the common experience of breeders whose observations, unfortunately, are too often unreliable. There are not wanting experiments such as those of Van Guaita ('98) and Bos ('94) and others, scientifically conducted, which bear out this conclusion.

On the other hand, it is refreshing to encounter in the literature such reports as that of Gentry ('05) who believes not only that inbreeding is not necessarily harmful, but also that it may be beneficial to conserve and intensify the good points in his breed. Gentry's experiments were made on Berkshires. The most prolonged tests of close inbreeding that have been recorded were made by Castle ('06) on the same species with which the present paper deals. He inbred (brothers with sisters) for fifty-nine generations. He concludes that such close inbreeding does not necessarily result in a loss of productiveness and of vigor; at least that inbreeding cannot be regarded as a causal factor. Some of his results so nearly parallel those of the present writer that further reference to his results will be made in the body of the paper.

During the early part of October, 1903, a number of pairs were started breeding. These came from various sources in Bloomington. These different pairs were reared for the most part only a few generations, excepting pair No. 6 which was continued for about four and one-half years. During this time over seventy-five generations were produced. Toward the close of this period no exact count was kept of the generations so that only an approximate figure can be given. Five pairs of brothers and sisters were mated in each generation to insure against accidents that might terminate the strain if but one mating were made.

Along at the fifth and sixth generation it became more and more difficult to keep the strain alive with the five pairs of brothers and sisters that were mated each generation. The failure of an

occasional pair to produce young had hitherto been attributed to accidental conditions of food, etc., but this no longer seemed a satisfactory explanation of all the failures to produce young. This condition, was, therefore looked into more thoroughly. This was done by laying out instead of five pairs a much larger number from the offspring of a given productive pair. The greatest care was taken with the food, temperature etc. and it soon developed that a variable per cent of the pairs were sterile. These sterile pairs were to all appearances normal. It was clear now that, while inbreeding had not reduced the general vitality of the strain thus far, there had appeared a high degree of sterility.

## 2. Sterility

*a. Character of the sterility.* Examination of all the matings brought out the fact that in all cases eggs were present in large numbers. This seemed to suggest that the difficulty lay in the larvae either failing to emerge from the egg envelope or, succeeding in this, failing to carry themselves through the feeding stage or the transformation.

By a careful search of the food of the sterile pairs, after sufficient time for the larvae to mature had been allowed, it became evident that the difficulty lay at a time earlier than the pupal stage for none of the latter could ever be found. The food supplied these sterile pairs was the same as that of the fertile ones since it could not be foretold which pairs were going to prove infertile. Furthermore, the infertile pairs were usually kept for from twenty to thirty days, the best of food being supplied them from time to time. The same search showed that no larvae were present, at least so far as direct inspection of the food under a dissecting microscope could be depended upon.

It was always possible, of course, that the larvae failed to carry their development very far, and, thus, being small when they first emerge from the egg, might have been overlooked. It became necessary, consequently, to take the eggs as they were laid from time to time and keep them under observation to see whether the larvae ever emerged. This was done by placing a piece of banana

in the vial with a sterile pair and from time to time removing the eggs one by one with the point of a needle and placing them on a piece of moist filter paper in a separate vial. Usually twenty were placed in each vial and some food added for the larvae, should they emerge. Inspection of the eggs after twenty-four, forty-eight and seventy-two hours would readily reveal the number of eggs that had produced larvae. I have laid out thus at a great expense of time literally thousands of eggs from many infertile pairs, in many cases all the eggs that a given pair produced during the first twenty-five days of its life, but I have never seen a single egg that had hatched. Eggs of fertile pairs thus laid out will readily hatch so that all the larvae will have taken to the food twenty-four hours after the eggs are deposited.

Such infertile pairs copulate frequently and it would seem that impregnation should follow. I have never sectioned the eggs to see whether spermatozoa enter the eggs or whether they contain partially developed larvae which fail to hatch. I have, however, been able to determine in this strain which of the sexes is at fault. This was done in the following manner. After a pair by sufficient trial had proven itself infertile, the male was mated to a virgin female of a fresh strain that had not been inbred and possessed a high degree of fertility, and the female was similarly mated with a male, usually one whose fertility had been established. Sixty-four such cases were tried and in no case did the females fail to produce young and in no case did the males produce any although repeated copulations took place. It is evident from the foregoing, that, in this strain, the sterility lies exclusively in the male and that the female has lost, apparently, nothing in fertility. Castle (p. 735) reports, on the other hand, that either sex may be sterile. However, Castle took no account of the eggs and larvae but merely the production of pupae, so that his sterility cannot be with certainty compared to mine. It would seem, however, that in some strains infertility may be strictly confined to the males and in others to both sexes. That sterility is complete for all males, when it occurs, is shown by both our results.

*b. Degrees of sterility.* The foregoing experiments concerned themselves with such pairs as were completely sterile. Other pairs

of brothers and sisters from the same parents, however, were fertile. Judging from the productiveness of these, there was often a wide divergence. It would seem that, as a result of inbreeding, we had a condition of fertility ranging from absolute infertility to comparatively high fertility among the different pairs of brothers and sisters from any given pair of parents. To test this the following experiment was carried out: About two-hundred eggs from each of fifteen pairs of flies were laid out after the fashion indicated above. Ten of these pairs had been inbred for seventeen generations while five belonged to fresh stock that had not been inbred. Of the ten pairs of the inbred strain, five belonged to a strain which had arrived at a very low degree of fertility, namely only 36 per cent of the forty-two pairs tested were fertile (table 3, seventeenth generation, strain, A). These five pairs were brothers and sisters to many of the sterile pairs considered in the preceding section.

The other five pairs (of the ten inbred) were from a strain which had been held by selection to a high degree of fertility, namely 97 per cent of the thirty-four pairs tested were fertile. Both of these strains were descended from common great grandparents (table 3, seventeenth generation, strain B).

We have, thus, for comparison three conditions, namely, (1) eggs from a highly infertile inbred strain; (2) eggs from a highly fertile inbred strain; and (3) eggs from a presumably normal strain that had not been inbred. It should be added that the five pairs were taken at random and were not selected. Approximately the first two-hundred eggs of each pair were laid out in batches of about twenty to twenty-five to the vial. The number of eggs that hatched was noted in each case and also the number that emerged as imagos. Table 1 gives the summary of results.

From this table it appears that from the eggs which were taken from the inbred pairs with low fertility practically as large a per cent (97.27) hatched as from the eggs that came from the inbred pairs that showed a high fertility (98.2). The same is true in regard to the number that produced imagoes, 86.8 per cent and 85.1 per cent respectively. The fact clearly brought out here is that when infertility arises in this strain it arises suddenly and

does not present all intergradations. In other words, one does not find that among a large number of brothers and sisters some pairs whose eggs only partially hatch and other pairs that range in this respect, on the one hand, to perfect fertility and, on the other, to complete sterility. The fertility is either completely lost or it is of a high degree. Furthermore, when we compare the inbreds with the normals (not inbred) in regard to the percentage of eggs hatched no essential difference is observable. It would seem, therefore,

TABLE 1

*Inbred (low fertility)*

PAIRS	NUMBER OF EGGS PLACED	NUMBER OF EGGS HATCHED	NUMBER OF IMAGOS EMERGED	PER CENT OF EGGS HATCHED	PER CENT OF IMAGOS EMERGED
A.....	193	184	160	95.3	82.9
B.....	200	188	169	94.0	84.5
C.....	201	197	182	98.0	90.5
D.....	198	198	180	100.0	90.9
E.....	123	123	104	100.0	84.5
Total.....	915	890	795	97.27	86.8

*Inbred (high fertility)*

A.....	201	198	182	98.5	90.5
B.....	173	172	156	99.4	90.1
C.....	204	200	161	98.0	78.9
D.....	197	193	165	97.9	83.7
E.....	175	169	145	96.5	82.8
Total.....	950	932	809	98.2	85.1

*Normals (not inbred)*

A.....	215	211	193	98.1	89.7
B.....	70	70	48	100.0	68.5
C.....	153	152	132	99.9	86.2
D.....	224	218	144	97.3	64.2
E.....	158	155	144	98.1	91.1
F.....	146	127	109	87.7	74.6
G.....	223	222	205	99.9	91.9
Total.....	1189	1155	975	97.2	82.0

that the pairs that had not completely lost their fertility, in so far as hatching their eggs is concerned, had suffered no deterioration whatever as a result of seventeen generations of closest inbreeding.

A fact of further importance brought out by table 1 is that of the percentage of eggs that successfully produced imagos. This does not differ essentially in the two groups of inbreds nor do these differ essentially from the normals. Castle used as his measure 'productiveness,' meaning thereby the number of pupae that were successfully produced. Making allowance for some pupae which do not emerge, the imagos produced in my experiments were an approximation to his 'productiveness.' Inbreeding, consequently, does not affect adversely the productiveness of pairs that show any fertility at all.

Castle found that his strains showed an annual fluctuation in productiveness, the period of least productiveness falling in the late autumn and early winter. My own experiments extended over about four and one half years and, although I have been on the lookout for this, I have never observed it. As Castle himself suggests, this fluctuation was probably a function of the temperature of the room. My flies were kept in a room which varied from 60 to 80 degrees and, when this was not possible, they were placed in an incubator kept at about the same range of temperature. It may also be that the productiveness of his strain ran low at this time of the year because they were placed in new hands at the opening of the college year. My observation has been that it takes some time for a new man to learn all the conditions that make for a favorable rearing of these creatures so that Castle's low productive periods may be merely a measure of the training period of the experimenter.

### *3. Inbreeding and vigor*

At the outset of the experiments it was the expectation of the writer that such rigorous inbreeding would early and violently show itself in the vigor and fertility of the animals. In this, however, he was largely disappointed. In the strain that is here under consideration no untoward results could be detected during the

first five or six generations. As previously stated, up to this time the method consisted in placing pairs of brothers and sisters in each of five vials to insure against mishaps. These mishaps consisted of drying up of the food, attacks of fungus and in some cases the escape of the flies themselves during the process of feeding etc. Those pairs that produced young were regarded as having escaped these various possible mishaps and were taken as indications of the vitality and productiveness of the strain. The expectation at that time was that any deleterious effect of the inbreeding would show itself in the offspring of any of the pairs. Consequently, when a given pair would produce offspring that was numerous, all well formed, vigorous, and in no apparent way differing from normal offspring, to see whether some slight influence might not be present that could not be detected by ordinary observation a definite measure was taken of (1) their rate of reaction to light and gravity, (2) the total number of eggs produced and (3) the percentage of eggs which hatched and emerged. An attempt was made to determine their length of life but this proved too prolonged to allow one to carry it out together with all the other incidents of the already too laborious experiments.

The reaction of this animal toward light and against gravity is well known. To get a measure of the rate of reaction the animals were made to travel through a glass tube that had been blackened for 16 cm. on the inside. This tube had a light placed at one end and was inclined about twenty-five degrees. From a glass vial the flies were admitted, one at a time, into the tube and the time from the moment of entrance into the blackened portion of the tube to their emergence was recorded. It was found essential that the two batches of flies (inbreds and normals) should be of the same age, be reared under the same conditions and that the temperature of the room be the same for the two batches. The results are as follows: at a temperature of  $27.2^{\circ}$  C. 133 normals took 16 seconds, average, to travel the distance, and 140 inbreds took 15.4 seconds. The two sexes in these two groups were about equal in number. In both groups the males travel the distance on an average in three seconds less time. It is clear from this that the normals and inbreds are equally responsive to these two

agents and that the latter have not suffered in this regard as a result of inbreeding.

In order to determine the total number of eggs produced it was necessary to isolate the pairs and twice each day pick off all the eggs that had been deposited in and around the food provided. This proved to be a most laborious process, for the eggs are too small to be followed safely with the naked eye and had to be removed individually with the point of a needle. Too much value must not be attached to this measure for the reason that the rate and, therefore, probably the number of eggs deposited seems to depend somewhat, at least, on the condition of the food present, and for the

TABLE 2

*Strain 6*

Number of generations inbred.....	2	3	5	6	8	9
Number of days eggs were counted.....	27	30	34	34	23	32
Total number of eggs laid.....	433	617	480	724	455	516

*Strain 7*

Number of generations inbred .....	2	3	5	6	9	10
Number of days eggs were counted.....	26	33	29	23	33	28
Total number of eggs laid.....	654	662	539	498	907	429

reason that only the eggs deposited during the first twenty-five or thirty days were counted. These creatures live to be very much older. We have kept females alive 153 days, but after the first twenty-five or thirty days the eggs come only in small numbers. Table 2 gives the actual counts of several females of both strains 6 and 7.

We see from the above counts that no material reduction has occurred in egg production during nine and ten generations of inbreeding. Such variations as occur may, of course, represent individual differences in the females.

The data given in table 1 of the relative hatching and emerging qualities of the young of normals and of pairs inbred for seventeen generations shows that there is no difference in this respect.

In so far as the above determination may be taken as a measure of the vitality of this species we are justified in concluding that from six to seventeen generations of inbreeding no appreciable deterioration has resulted. No such exact determinations were made in later generations, and it is possible that eventually the effects of inbreeding would manifest themselves, but my observations during seventy-five or more generations does not lead me to believe this.

#### *4. Sterility and selection*

Along at the thirteenth and fourteenth generations the sterility had become very pronounced. Of the offspring of some of the pairs, more than 50 per cent of the males were sterile. On the other hand, while practically all pairs showed at least some degree of sterility this varied very much in the different brothers and sisters of the same brood. That this sterility was a direct physiological result of the inbreeding seemed to me very doubtful. To find the effects of inbreeding showing itself in such a specific way upon the males only, did not, to say the least, meet expectations. Furthermore, sterility was not wholly wanting in forms that had not been inbred.

It was highly desirable to continue the experiments on inbreeding, and yet to keep the strain alive, it was necessary to find some way to eliminate this high degree of sterility. The process that was most effective was selection. By continuing the strain of those pairs whose offspring showed the highest degree of fertility but at the same time continuing the rigorous inbreeding, it was possible almost completely to eliminate the sterility. This at the same time gave one of the severest tests as to whether inbreeding was the responsible factor, for if the sterility could be eliminated by continuing the very process of inbreeding the latter could not well be held to be the cause of it.

This was done as follows: In the fourteenth generation three fertile pairs of brothers and sisters from the same brood were isolated and mated. The offspring of each of these were mated in pairs to determine the degree of sterility. By reference to table

TABLE 3  
*History of Strains A, B and C*

Generation

Generation

6,14,17 (From brood with 84.6% fertility)

Generation 14: A (1), B (1), C (1)

Generation 15: F (3) (25%), I (9) (75%), F (10) (71%), I (4) (29%), F (10) (67%), I (5) (33%) (Discontinued)

Generation 16: F (20) (43%), I (27) (57%), F (37) (100%), I (0)

Generation 17: F (15) (36%), I (27) (64%), F (33) (97%), I (1) (3%)

Generation 18: (1) 15 (100%), (2) 14 (28%), (3) 37 (71%), (4) 45 (80%), (5) 19 (67%), (6) 9 (33%)

Generation 19: (1) 27 (53%), (2) 23 (47%), (3) 39 (77%), (4) 44 (85%), (5) 24 (69%), (6) 11 (31%)

Generation 20: (1) 37 (90%), (2) 4 (10%), (3) 42 (93%), (4) 33 (91%), (5) 42 (95%), (6) 2 (5%)

3, it will be seen that the pair marked *A* produced offspring out of which nine of twelve pairs tested were infertile; pair *B* produced offspring of which four pairs out of fourteen tested were infertile and pair *C* threw offspring with five pairs out of fifteen infertile. We have here, then, three pairs showing a wide variation in the degree of fertility of their offspring. Pair *A* showed 75 per cent of the pairs infertile and pairs *B* and *C* approximately the reverse ratio. In the further progress of the experiment pair *C* was dis-

TABLE 4

*Strain A*

	NUMBER PAIRS TESTED	NUMBER PAIRS FERTILE	NUMBER PAIRS INFERTILE	PER CENT PAIRS FERTILE	PER CENT PAIRS INFERTILE
18 (1).....	52	27	25	51	49
18 (2).....	51	37	14	72	28
18 (3).....	52	37	15	71	29
18 (4).....	56	45	11	80	20
18 (5).....	28	19	9	69	31

Average for 238 pairs 69 per cent.

*Strain B*

	NUMBER PAIRS TESTED	NUMBER PAIRS FERTILE	NUMBER PAIRS INFERTILE	PER CENT PAIRS FERTILE	PER CENT PAIRS INFERTILE
18 (1).....	15	15	0	100	0
18 (2).....	14	14	0	100	0
18 (3).....	19	19	0	100	0
18 (4).....	22	15	7	68	32
18 (5).....	23	23	0	100	0

Average for 93 pairs 92.5 per cent.

continued so that only pairs *A* and *B* were used. I shall in the further description of the experiment refer to the descendants of *A* as strain *A* and of *B* as strain *B*.

Before entering upon the experiment of selection it was necessary to ascertain whether, without selection, the descendants of pairs *A* and *B* continued to show a low and high fertility respectively. Accordingly, a single one of the fertile pairs of the 15th inbred generation of strain *A* and *B* was tested. Reference to the table shows that in strain *A* 27 pairs or 57 per cent of the forty seven pairs tested were infertile, while in strain *B* none of the thirty-

seven pairs tested were infertile. The same process was repeated with a pair of the sixteenth generation of the two strains. Strain *A* showed twenty-seven or sixty-four per cent of the forty-two pairs tested infertile and strain *B* one or three per cent of the thirty-six pairs tested.

Up to this point in the experiment only a single pair in each generation was tested as to the fertility of its offspring. It might well be that by chance in each case a pair of low fertility was taken in strain *A* and a pair of high fertility in strain *B*. To eliminate this possible error five pairs were taken in each strain and the fertility of their offspring determined. It was further desirable to obtain an estimate of the variability in the fertility of the pairs in the two strains as well as to get a more correct estimate of the average fertility of both. In the diagram these five pairs are designated as 18 (1), 18 (2), etc. Table 4 shows the number of pairs of offspring tested for each pair and the number and percentage of pairs fertile and infertile.

The fertility thus varied in strain *A* from 51 per cent in 18 (1) to 80 per cent in 18 (4), with an average fertility of 69 per cent. In strain *B* the fertility was much less variable in the different pairs, the only exceptions being 18 (4), the average fertility being 92.5 per cent.

We now have definitely established two strains, one of low and another of high fertility. The important part to be emphasized here is that this was produced by the process of selection from among the variable offspring of generation fourteen of the inbred strain. To make the experiment more complete it was now necessary to obtain a highly fertile strain out of the one with low fertility. Accordingly strain *B* was discontinued at this point and attention restricted to strain *A*. Five pairs, 19 (1), 19 (2), 19 (3), etc., were taken from among the offspring of 18 (4) because this showed the highest percentage of fertility. These were tested in the same way as in the preceding generation. Table 5 gives the details.

By selection it will be seen that the average fertility was raised from 69 per cent in the 18th generation to 75 per cent in the 19th generation. Among the five pairs used one 19 (2) showed an unusually high fertility (96 per cent). This pair was accordingly

TABLE 5

	NUMBER PAIRS TESTED	NUMBER PAIRS FERTILE	NUMBER PAIRS INFERTILE	PER CENT PAIRS FERTILE	PER CENT PAIRS INFERTILE
19 (1).....	50	27	23	53	47
19 (2).....	51	49	2	96	4
19 (3).....	51	39	12	77	23
19 (4).....	52	44	8	85	15
19 (5).....	35	24	11	69	31

Average fertility of 239 pairs 75 per cent.

taken to select from. Five pairs were again taken as before. The results appear in table 6.

Thus it will be seen that all five pairs showed a uniformly high degree of fertility. The average fertility of all the pairs was raised to 93.8 per cent.

### 5. Discussion

From the above series of experiments a number of important facts are brought out. 1. Sterility, as it appeared in the strain under consideration, is strongly transmissible through inheritance. 2. It is readily controlled by selection. 3. Inbreeding is probably not the physiological cause of it.

That this sterility is transmissible cannot be doubted. The faithfulness with which this occurs appears in the strains *A* and *B*. Both were derived from a common pair that showed a variability with respect to this character in the three pairs of its offspring

TABLE 6

	NUMBER PAIRS TESTED	NUMBER PAIRS FERTILE	NUMBER PAIRS INFERTILE	PER CENT PAIRS FERTILE	PER CENT PAIRS INFERTILE
20 (1).....	41	37	4	90	10
20 (2).....	45	42	3	93	7
20 (3).....	45	44	1	97	3
20 (4).....	36	33	3	91	9
20 (5).....	44	42	2	95	5

Average for 211 pairs 93.8 per cent.

tested. One of these possessed a high degree of sterility, while the two other pairs showed a low degree. The descendants of the latter constituting strain *B*, retained this low degree of infertility throughout. Similarly the descendants of the former, constituting strain *A*, retained their high degree of infertility up to the time when selection away from this condition was introduced. In the latter process the transmissibility of the character is again emphatically revealed. In the eighteenth generation, pair 4 showed a lower degree of sterility than any of the remaining four pairs of brothers and sisters. Breeding from this pair at once showed offspring with a decided decrease in sterility, compared with the eighteenth generation, the average of the nineteenth generation being 75 per cent of the pairs fertile as compared to 69 per cent of the latter. Again, in the nineteenth generation, pair 19 (2) showed a much lower degree of infertility than the other pairs. Continuing the strain from this pair, this character is faithfully reproduced in the offspring in that the average fertility of the latter is raised to 93.8 per cent.

It is important to note in this connection that Castle, in his experiments upon *Drosophila*, found that productiveness (which as previously noted is quite a different thing from the sterility here considered) was similarly transmissible and amenable to selection. Furthermore, Castle's experiments would seem to indicate that this character of productiveness behaves, in inheritance, after the Mendelian fashion, low productiveness acting as the recessive character. We have evidently to do here, both in the productiveness in Castle's experiments and in the sterility in my own, with characters that are germinal for they behave as such. In the strain upon which my experiments were made we have the further remarkable condition that the infertility is inherited only by the males.

It is clear that whatever the causal factor or factors to which the sterility may be attributed, it is relatively insignificant compared to the effect of selection upon it. Furthermore, the modification is a germinal one. That inbreeding may be responsible for its prevalence in the strain seems probable, but that it is responsible

for its origin is not believed. We have seen that the general vitality of the strain, as measured by its productiveness and its reaction to light and gravity, did not suffer as a result of seventeen generations of closest inbreeding. Failing in this, it is not probable that its effect would show itself in so specific a way as the sudden and complete sterility in certain males of the strain. The improbability is further supported by the fact that the inbreeding may be continued unabated if only care be exercised in the selection of the brothers and sisters to be mated, thereby even eliminating practically what sterility may have existed.

It is much more probable that the sterility arose spontaneously in this strain or that it is present to a varying degree in this species. With the character present and highly transmissible and subject to selection it is only necessary to carry on indiscriminate breeding to have the character appear in varying intensities depending upon the chance combinations. The rule of inbreeding would be only to intensify the chance combination of the character and to insure the more or less continued presence in the successive generations.

That this character of sterility is not unique to this inbred strain is evident from its rather frequent presence in pairs not inbred. In my own experience this sterility nearly always showed itself in the males. In one instance I found among a brood, besides a sterile male, two females that failed to deposit eggs although eggs were evidently present in the oviducts. Similarly Castle found in his strain a considerable amount of sterility, and this in some cases among the females. We see, therefore, that sterility is not altogether rare even in broods that were not inbred.

The same facts doubtless hold for the character of productiveness. Castle has shown this to be transmissible and amenable to selection. Inbreeding does not produce it but is instrumental, with indiscriminate mating, in intensifying it, or, if the strain be not eliminated thereby, of preserving it in the strain.

## SEX-RATIO AND SELECTION

*1. Introductory*

The once rather generally accepted notion that nutrition was an influential factor in the control of sex, based on the experiments of Yung ('85), Born ('81), and others, has given place to the now as commonly accepted idea that sex is determined prior to or at the time of fertilization and is independent of the food. The experimental work of Cuénot ('99) King ('07) and others, and the splendid cytological researches of Wilson and his students are largely responsible for this change of view and have been so frequently reviewed in the various recent discussions of the problem of sex that they need not be further detailed here.

The writer tried some starvation experiments on *Drosophila* in 1904. During the past year more extensive experiments were carried on under his direction by Mr. Claude D. Holmes, on the effects of starvation during successive generations upon the sex-ratio. These are published under a separate title ('10). It will suffice in this connection, to state that the results coincide with those of recent workers, namely that nutrition does not affect the sex-ratio.

*2. The normal sex-ratio*

One fact was very apparent in these earlier tests and in all subsequent experiments, that, under the varying conditions in these creatures were reared, there was the same persistence of the predominance of females over males. Below (table 7) is given the

TABLE 7

FOOD	TOTAL NUMBER REARED	NUMBER OF MALES	NUMBER OF FEMALES	RATIO
Bananas.....	10506	4972	5534	1:1.113
Grapes.....	2161	995	1166	1:1.171
Tomatoes and grapes.....	4048	1943	2105	1:1.083
Bananas.....	10218	4757	5461	1:1.14
Total.....	26933	12667	14266	1:1.126

summary of four determinations on a large scale to obtain the normal sex-ratio. The flies were reared in the following manner. Mason jars containing a large quantity of food were exposed to flies in nature. The jars were left open until the larvae began to pupate when all flies were excluded by tying a guaze over the top. As the imagos emerged from time to time they were preserved and the sex-ratios determined. For 26933 individuals, the ratio was one male to 1.126 females.

In regard to these determinations only one question, so far as I can see, can be raised. This is the academic one of the greater mortality of the males during development or, to push the matter back a little further and to make it applicable to recent developments in our idea of sex, the greater mortality of the male determining sex cells. In reference to this it may be pointed out that the developmental conditions were as nearly normal as one can imagine. There was an abundance of food, air, light and moisture, and the larvae pupated in the remnants of the food in much the same manner as one finds them doing in nature. In this connection the experiments of Miss King ('07) on the influence of food on the sex ratio of *Bufo* are of importance. In this she finds that the mortality among the males is not greater than among the females. From these facts and from the knowledge that has come to me from the extensive rearing of *Drosophilas* for six years I am convinced that the sex-ratio in this species is not one of equality.

### *3. Control of sex-ratio by selection*

If the sex-ratio of this species, then, is that of 1 male to every 1.126 females, this should be regarded as specific just as any other of the specific characters of the species. It should, therefore, be subject to fluctuations and to control like other specific characters.

Starting with this conception of sex-ratio, I wished to see whether it were possible to control this, within limits, of course, by the process of selection. The results of these experiments I propose to detail below.

To apply the selective process on the sex-ratio, the following simple method was employed. Two pairs were selected from

nature, the one showing a high, the other a low female ratio. These were selected as the parents of the two strains to be developed. From among the offspring of each of these two pairs a number of single matings were made. From among these the pair that showed the most favorable ratio in the desired direction was selected to continue the strain. The same process was repeated as often as desired.

From a number of pairs taken from a banana bunch in Bloomington June 12, 1907, two such pairs were obtained. These two pairs go by the numbers 206 and 207, showing the following ratio:

206—52 ♂: 135 ♀ or 1:2.59

207—84 ♂: 75 ♀ or 1:0.89

A. *Strain 206 (high female ratio)*. The 206 strain will, for convenience, be called the female strain and the 207 strain the male strain, although, as will appear, the latter never developed into a predominantly male strain. In tables 8 and 9 are given in diagrammatic form the results of selection for five generations in the former and six generations in the latter. At the margin the generations are numbered 1, 2, 3 etc., and the sex-ratios are indicated.

The sex-ratio of the eleven pairs of brothers and sisters mated from the first generation of the female strain (206) varied from 1:93 (76 ♂; 71 ♀) to 1:7.00 (8 ♂: 56 ♀).

The unusually high female ratio in the latter is probably attributable to the small number of individuals obtained from this pair. Two of the pairs threw a predominance of males (table 8 nos. 4 and 8). With the exception of no. 5, all the remaining pairs threw a high female ratio. The ratio for all the pairs was 1:1.67 (578 ♂: 969 ♀). We have here a female ratio very much higher than that characteristic of the species (1:1.14) and yet considerably below that of the parent pair (1:2.59). This may be regarded as a regression toward the normal ratio. It should be pointed out here that too much emphasis should not be placed upon the exact figures representing the ratios in the different pairs, since the number of individuals at best are rather small. In most cases, however, when the number of offspring obtained is fairly large, the ratio approximates the true one, so that in any given





pair from which a fairly large number of offspring has been obtained shows a high female ratio for instance, this may be taken as a pretty safe indication that the female ratio would be high if all or a much larger number had been obtained.

For the next generation ten pairs were taken from brood 9 with a ratio of 1:1.94. Brood 3, with a ratio of 1:2.31, would have been a more favorable one to select from, but this is not always possible since the matings must be made before all the offspring have emerged and therefore all the data for the complete ratio is obtained. Only four pairs of this series of matings came through safely, due purely to the lack of time to give them the attention they should have had. The four pairs threw the following sex-ratios: 1: 2.33; 1:2.29; 1:1.27; 1:1.81. The ratio for the entire brood was 1:1.82 (215 ♂: 391 ♀). This ratio was somewhat more predominantly female.

Pairs were now selected from the brood 8 with a ratio of 1:2.29. Of the seven pairs mated the offspring of only four was obtained and the number of young in each case was quite small. The ratio for all the offspring of the generation was 1:2.17 (93 ♂ to 201 ♀). The total number here involved is so small that not too much importance should be attached to the increased female ratio.

For the matings of the next generation there is little doubt that an unfortunate selection was made. The brood from which the matings were taken showed a ratio of 1:2.46 but this ratio was based on numbers so small (52) that it probably did not represent the true ratio of the pair. This may account for the drop in the ratio for all the broods of the 4th generation to 1:1.36 (354 ♂ 483 ♀).

Two sets of matings were now made from as many broods of the fourth generation. One of these series was again taken from the brood showing the most favorable female ratio 1:1.90 (85 ♂ 162 ♀), but the other series was taken from a brood showing a relatively low female ratio, 1:1.04 (64 ♂ 67 ♀). From the former the ratio of five pairs was obtained showing a ratio of 1:1.39 (372 ♂—518 ♀) and from the latter the ratio of 7 pairs, showing a ratio of 1:1.07 (496 ♂: 535 ♀).

*b. Strain 207 (low female ratio).* From pair 207 with a ratio of 1:0.89 (84 ♂: 75 ♀) it was hoped to develop by selection a strain showing a low female ratio. Seven matings from the first generation produced 536 ♂ and 579 ♀, or a ratio of 1:1.08. The range of ratios of the individual pairs was from 1:1.22 (99 ♂: 121 ♀) to 1:0.86 (79 ♂: 68 ♀). This selection was continued for four generations, the matings being made from broods with a low female ratio. The ratios of all the offspring in the successive generations were 1:1.06 (220 ♂: 223 ♀) 1:1.10 (581 ♂: 640 ♀); 1: 1.04 (142 ♂: 147 ♀); 1:1.17 (518 ♂: 607 ♀) for the second, third, fourth and fifth generations respectively (See Table 9). This low female ratio showed itself rather uniformly in all the individual matings, a notable exception occurring in the fifth generation (see Table 9, pair 3.) with a ratio of 1:2.53 (45 ♂: 144 ♀). On the other hand no pairs threw a great preponderance of males, the most notable among those from which a large number of progeny was obtained being pair 2 in the third generation in which the ratio was 1:0.87 (115 ♂: 101 ♀). For the sixth generation two sets of matings were made as in the fifth generation of the strain 206. One of these was made from a brood with a ratio of 1:2.53 (45 ♂: 144 ♀) and the other from a brood with a relatively low female ratio, 1:1.36 (72 ♂: 98 ♀). From the former the total progeny of eight matings gave a ratio of 1:1.42 (461 ♂: 654 ♀) and from the latter the ratio of eleven matings was 1:1.05 (944 ♂: 997 ♀).

*c. Discussion.* It seems from the above experiment that the sex-ratio in this creature is a strongly transmissible character. Starting with a pair that throws an offspring showing either high or a low female ratio it was possible to maintain, by selection, a strain maintaining the respective ratios. The offspring from a given pair, when mated in pairs, show a considerable variation in the sex-ratio of their children. It is thus possible to develop a strain with a low female ratio from one with a high female ratio, or the reverse, as is shown in the fifth and sixth generation of experiment 206 and 207 respectively (tables 8 and 9). The sex-ratio is clearly amenable to selection like any other character.

It is an interesting fact that it is possible to develop a strain with a high female ratio much more easily and pronouncedly than a male strain. I have repeatedly tried to hold the sex-ratio to or below that of unity but without success. Not infrequently pairs will throw a predominance of males but it has not been possible to hold them there. The best I have ever been able to do is to hold it considerably below that of the normal, but never as low as unity. On the other hand, it is relatively easy to select in the direction of females even to the extent of 1 to 2.

It should be observed that in the breeding of these strains the most rigorous inbreeding was practiced. It might, therefore, be that the difficulty of selecting for a low female ratio results from the possibility that inbreeding tends toward the elimination of the males. My extensive experience in inbreeding these creatures, however, does not bear out this explanation. Furthermore, in the sixth generation of the high female strain it was possible in two generations to reduce this ratio to near unity notwithstanding that the same rigorous inbreeding was continued.

#### *4. Relative influence of male and female in determining the sex-ratio*

Having thus produced two strains showing a decided difference in the sex-ratio of their offspring I wished to determine two further points. First, whether the maternal or the paternal elements had an equal share in the control of this ratio, and second, whether this ratio was determined in the process of fertilization. To this end reciprocal crosses were made between the two strains and the proportion of the sexes in the offspring ascertained. Three experiments were performed in the following manner. From among a brood of each of the two strains a large number of individuals were taken. Before sexual maturity a number of males and females were isolated, while the remainder were allowed to reproduce. The latter gave a control for each of the strains. The isolated virgin females of one strain were mated with the males of the other. Each experiment thus consisted of four multiple matings. (1) A number of brothers and sisters belonging to the male strain. This furnished a control for the male strain. (2) A number of brothers and sisters belonging to the female strain. This furnished a control

for the female strain. (3) Females from the male strain mated with males from the male strain, and (4) the reciprocal of '(3)'.

In crossing two strains as in the above experiment three possibilities might obtain. First, that the two sexes have an equal influence in determining the sex-ratio; second, that either sex have a predominant influence and third, that a ratio result unlike that obtaining in either of the parental strains. While the first is probably the expected result, the experiments show in a most decided way that the male has little or no influence in determining the sex-ratio in this species (tables 10, 11 and 12). In most of the cases the ratio of the offspring falls pretty closely around that of the strain from which the females were taken. In two instances the ratios exceeded 100 per cent influence. The remaining ones, with the exception of strain 244 in which the male influence amounted to 35 per cent show the female influence almost near enough to 100 per cent to justify one in regarding the differences merely as fluctuations incident to the small number of individuals involved. The unusually great influence of the male in strain 244 might be accounted for in two ways. First the number of individuals involved in this experiment are relatively small so that the ratios of both the control and the crossed broods are not as reliable as in the other experiments. Secondly, the flies used for this experiment were taken from the earlier generations of the two strains, before, we may believe, any considerable selection had been applied to fix the character of the respective strains. Indeed, this seems to be borne out in the other experiments.

The materials of the three experiments were not all taken from the same generation but were taken from different generations in the development of the strain. Thus, in experiment 1 the broods were taken from the first generation of strain 206 and 207. In experiment 2 the broods came from the second generation of strain 206 and the third of 207. The third experiment was made from the fourth and fifth generations of strains 206 and 207 respectively. Arranging these experiments in a series, based on the length of time that selection had been practiced on the broods used, we see that the male influence decreases as the selective time increases.

TABLE 10

*Experiment 1*

No. of strain mated..... {	No. 242 212 <sub>2</sub> × 212 <sub>2</sub>		No. 245 212 <sub>2</sub> ♀ × 214 <sub>9</sub> ♂		No. 243 214 <sub>9</sub> × 214 <sub>9</sub>		* No. 244 214 <sub>9</sub> ♀ × 212 <sub>2</sub> ♂	
	♂	♀	♂	♀	♂	♀	♂	♀
Number of individuals.....	208	194	463	475	171	273	225	311
Sex-ratio (actual).....	1.00	0.98	1.00	1.03	1.00	1.60	1.00	1.38
Theoretical ratio.....			1.00	1.288			1.00	1.288
Influence of male parents....	7.3 per cent				35 per cent			
Influence of female parents..	92.7 per cent				65 per cent			

TABLE 11

*Experiment 2*

No. of the strains mated.. {	No. 271 252 <sub>10</sub> × 252 <sub>10</sub>		No. 274 252 <sub>10</sub> ♀ × 255 <sub>8</sub> ♂		No. 272 255 <sub>8</sub> × 255 <sub>8</sub>		No. 273 255 <sub>8</sub> ♀ × 252 <sub>10</sub> ♂	
	♂	♀	♂	♀	♂	♀	♂	♀
Number of individuals.....	332	545	589	919	739	818	680	698
Sex-ratio (actual).....	1.00	1.69	1.00	1.56	1.00	1.106	1.00	1.026
Theoretical sex-ratio.....			1.00	1.365			1.00	1.365
Influence of male parents....	22 per cent				0 per cent (—13)			
Influence of female parents..	78 per cent				100 per cent (1.13)			

TABLE 12

*Experiment 3*

No. of strain mated..... {	No. 279 275 <sub>8</sub> × 275 <sub>8</sub>		No. 281 275 <sub>8</sub> ♀ × 278 <sub>7</sub> ♂		No. 280 278 <sub>7</sub> × 278 <sub>7</sub>		No. 282 278 <sub>7</sub> ♀ × 275 <sub>8</sub> ♂	
	♂	♀	♂	♀	♂	♀	♂	♀
Number of individuals.....	289	427	382	551	1022	1044	752	825
Sex-ratio (actual).....	1.00	1.477	1.00	1.50	1.00	1.021	1.00	1.083
Theoretical ratio.....			1.00	1.249			1.00	1.249
Influence of male parents....	0 per cent				13 per cent			
Influence of female parents..	100 per cent				87 per cent			

TABLE 13

		PER CENT OF FEMALE INFLUENCE	PER CENT OF MALE INFLUENCE
Experiment 1-from broods selected for one generation.....	$\left( \begin{array}{cc} 212 & 214 \\ 214 & 212 \end{array} \right)$	$\left( \begin{array}{c} 92.7 \\ 65 \end{array} \right)$	$\left( \begin{array}{c} 7.3 \\ 35 \end{array} \right)$
Experiment 2-from broods selected for 2 and 3 generations.....	$\left( \begin{array}{cc} 252 & 255 \\ 255 & 252 \end{array} \right)$	$\left( \begin{array}{c} 78 \\ 100 \end{array} \right)$	$\left( \begin{array}{c} 22 \\ 0 \end{array} \right)$
Experiment 3-from broods selected for 4 and 5 generations.....	$\left( \begin{array}{cc} 275 & 278 \\ 278 & 275 \end{array} \right)$	$\left( \begin{array}{c} 100 \\ 87 \end{array} \right)$	$\left( \begin{array}{c} 0 \\ 13 \end{array} \right)$

This fact of the prevailing or exclusive influence of the female in determining the sex-ratio occurs in some other species of animals. Phylloxerans (Morgan '09) and *Dinophilus apatris* (Korschelt '82). On the other hand, Whitney ('09) seems to have shown that in rotifers certain eggs which will produce males if unfertilized are changed to females, if impregnated. In the case of *Drosophila*, we can not be certain that the sex-ratio is established before fertilization since the experiments do not with certainty entirely exclude the male influence.

### 5. Discussion of sex-ratio

It is not the intention to enter into an elaborate discussion of the problem of sex control. The literature is certainly already sufficiently burdened with such. The writer wishes merely to point out briefly a few conclusions about sex in this species which his results seem to warrant.

The property of sexuality possessed by this species expresses itself not in the equal production, numerically, of its two states, male and female, but in an unequal production. Studies in normal sex-ratios involving a sufficiently large number of individuals are not numerous. The unequal production of the two sexes in the human species is well established. Montgomery ('08) has given the data of a large number of individuals of *Theridium* and finds a marked inequality in the sexes. The general assumption seems to be that an equal sex-ratio is the rule. It is not improbable,

however, that, as careful determinations upon different species multiply, the condition of unequal ratios will be found increasingly common. Any theory of sex must take into consideration this normal inequality in the sex-ratios.

Sex-ratio like color, size etc., is a character belonging to a species. Sexuality of course is not, for it is common to all species reproducing by the sexual method. The particular form of sexuality, however, the proportion of the two sexual persons to which it gives expression in the process of differentiation, this is specific. For *Drosophila ampelophila*, the ratio of one male to 1.126 females is a specific character. This is not a ratio of merely the present generations but has been transmitted from generations remote. It is inherited. It is the expression of the physiological condition to which the species has been developed by its environmental demands.

Like other specific characters this ratio should be subject to modification, but this should not be more easily done or by other methods, in general, than those used in the modification of other characters. From this view point it should not be expected that the sex-ratio in an animal could be materially changed by such agents as food, temperature, etc. A change in the proportion of the sexes involves a much more fundamental modification than simple starvation or the reverse is likely to induce. In regard to other characters, we have long ago ceased to regard them as modifiable by such methods, but in the case of sex, it is only recently that their futility is being entertained. The most potent factor and the one most generally used to modify a character is selection. If the experiments herein recorded prove what they are held to prove, this process of selection is a potent factor in the modification of the sex-ratio also. It would be interesting to try to line this fact up with the chromosomal conception of sex. However, the writer regards this as the task of those who are engaged in these interesting and important investigations.

## SUMMARY

1. *Drosophila ampelophila* may be inbred (brothers and sisters) for seventy-five or more generations.

2. Inbreeding in itself is not deleterious to the fertility or vigor of this species.

3. Infertility normally occurs to a varying degree among the offspring of any pair. Promiscuous inbreeding among such offspring may perpetuate and even intensify this character. When sterility appeared in the strain experimented with, it was always complete, appeared suddenly and was confined to the male.

4. By the judicious selection of the brothers and sisters to be mated from a brood that shows a high degree of infertility, this infertility can be eliminated by selection although continuing the inbreeding in the closest possible way.

5. There is a wide divergence in the fertility and productiveness among the different pairs taken in nature, but by the proper selection and closest inbreeding these may be readily brought to either a high or low state with respect to these characters.

6. Many generations of closest inbreeding does not necessarily cause any loss in size, perfection of form, rate of reaction to light and gravity, egg production or length of life and sex-ratio.

7. The normal sex-ratio of this species in nature when reared under diverse conditions of food is one male to 1,126 females.

8. Different pairs in nature show a wide divergence in the sex-ratio of their offspring.

9. When the offspring from a pair with a given ratio are mated in pairs their offspring will show a wide range in the sex-ratio but in the aggregate will tend to reproduce the ratio of the brood to which they belong.

10. Sex-ratio is therefore a character that is strongly transmissible. By the proper selection of pairs tending to throw a high female ratio on the one hand or a low female ratio on the other it is possible to develop strains characterized by high or low female ratios.

11. In this species it is comparatively easy to develop a strain with a female ratio considerably higher than the normal but very

difficult to develop a strain with a female ratio much lower than the normal or even one in which the sexes are equal in number.

12. Sex-ratio is one of the qualities that is, like color, an inherent characteristic of this creature, strongly transmissible and amenable to the process of selection.

13. The female is almost wholly responsible in the transmission of the sex-ratio. For, if females from a strain possessing a high female ratio be mated with males from a strain possessing a low female ratio or vice versa, the offspring will show a sex-ratio which is wholly or very near that of the strains from which the females were taken.

14. Sex is probably very little, if at all, influenced at fertilization in this species, but is probably determined much earlier and by the female, but there seems no reason why this may not be influenced by various factors and in some species at fertilization.

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## THE MECHANISM OF LOCOMOTION IN GASTROPODS

G. H. PARKER

### INTRODUCTION

The snail's foot in locomotion is so striking and so easily observed that it has excited the interest of naturalists for a long time and yet a complete solution of even the mechanical problems connected with its action seems not to have been attained. Within recent times a number of investigators have attacked the problem of locomotion in snails, but their efforts have been directed chiefly toward the elucidation of the action of the neuromuscular mechanism rather than toward an understanding of the external mechanical conditions that accompany locomotion. It is the object of this paper to consider, in the light of the more recent investigations and from the standpoint of renewed observation, the external mechanical factors involved in the movements of the gastropod foot.

The observations recorded in this paper were made partly at the Bermuda Biological Laboratory, at the Harvard Zoölogical Laboratory, and at the Biological Laboratory of the United States Bureau of Fisheries at Woods Hole. I am under obligations to the directors of the laboratories mentioned for the materials and opportunities for carrying on these studies.

### TYPES OF MOVEMENT

When the locomotor movements of the foot in many species of gastropods are compared, a surprising diversity is found. These different types of movement have been well classified by Vlès

('07) and are apparently characteristic not only for species but for larger groups of gastropods. In the majority of species thus far examined, the pedal waves course forward over the foot, thus agreeing in direction with the animal's locomotion. Vlès has appropriately designated this type of movement as the *direct* type and has given the following gastropods as examples; the pulmonates (including *Onchidium*), *Aplysia*, *Aeolis*, *Doris*, *Haliotis*, *Trochus*, *Cyclostoma*, and certain small species of *Littorina*. I can confirm this statement for such of these molluscs as I have examined, namely, many pulmonates, including *Onchidium*, and I can add to this list *Crepidula fornicata*. In other gastropods the waves pass over the foot from anterior to posterior and this type has been designated by Vlès as *retrograde*. As examples he has given *Acanthochites fascicularis*, *Littorina littorea*, and *L. rudis*. Besides confirming Vlès' observation on *Littorina littorea*, I can add to this list *Dolabrifera virens* Verrill, *Tectarius nodulosus* Gmel., *Nerita tessellata* Gmel., and *Chiton tuberculatus* Linn. According to the observations of Jordan ('01, p. 99) *Aplysia* belongs under this head and not under that of the direct type as given by Vlès.

In both chief types of movement several subtypes can be distinguished as determined by the lateral extent of the pedal waves. In some gastropods each wave extends over the functional width of the foot and thus the foot is occupied by only a single series of waves. This subtype has been termed by Vlès *monotaxic*, and is exemplified by the pulmonates and chitons. In addition to these gastropods, *Dolabrifera virens* also has a monotaxic wave. In other gastropods the foot is functionally or even structurally divided along the median plane and exhibits a double system of waves, one right and the other left. This subtype has been designated *ditaxic* by Vlès and is exemplified by *Haliotis*, *Trochus*, and *Cyclostoma* among the direct types, and by *Littorina littorea* among the retrograde types. Besides confirming Vlès' statement as to *Littorina littorea*, I can add *Tectarius nodulosus* and *Nerita tessellata* as ditaxic gastropods. In *Tectarius* the waves on the two sides of the foot usually alternate and they are

so extensive that never more than two waves can be seen on one side of the foot at once. The foot, therefore, moves forward in alternate steps, first on the right side and then on the left, the motion resembling that of a person in a sack walk. In *Nerita* the wave begins anteriorly as a single wave whereupon it breaks and passes down the right and left sides of the foot to unite as one wave again at the posterior margin. These two conditions of *alternate* waves, as in *Tectarius*, and *opposite* waves, as in *Nerita*, will probably be found exemplified in other ditaxic gastropods. In certain small species of *Littorina* with direct movements, Vlès has described four parallel sets of waves, fulfilling the requirements of a tetrataxic subtype. This occurs, according to Vlès, only in connection with the retrograde type of movement. I have seen no example of it.

Among those snails that I have examined, one species, *Ilyanassa obsoleta* (Say), seems to find no place in Vlès' classification. This snail is a vigorous, active creeper. Its foot covers a large area compared with the size of its body. Anteriorly the foot is truncated and auriculate; posteriorly it is bluntly rounded. Its ventral surface is whitish, flecked over with irregular grayish splotches. In resting, the snail uses chiefly the posterior part of the foot, the anterior part being sometimes more or less withdrawn into the shell. In locomotion the anterior part seems to be the more active. Notwithstanding the fact that this snail is very easily observed in active creeping and that its foot is marked in a most favorable way for exhibiting wave-like movements, I have never been able to discover any evidence of such movements. When in locomotion, the whole foot seems to glide at a uniform rate over the surface of attachment such as that of a glass plate. Only along the anterior edge and over a small portion of the anterior ventral surface of the foot, can slight variations in the rate of movement be discovered and these variations are so local and scattered that they can in no sense be regarded as forming a wave. The movement of the foot of *Ilyanassa* has a most striking resemblance to that of the foot of a planarian in which cilia may be the chief motor organs, but on testing the foot

of *Ilyanassa* with carmine suspended in seawater, not the least evidence of cilia could be discovered. I therefore believe that *Ilyanassa* moves by a form of muscular activity that does not appear as pedal waves and it is not improbable that other gastropods will be found that have the same peculiarity. That Vlès recognized something of this kind may be inferred from his statement that no changes in color can be seen in the creeping foot of *Nassa*, *Buccinum*, *Aeolis*, etc., and that the direction of the waves in these instances can be judged only by the deformations produced at the edge of the foot. As Vlès makes no further mention of *Nassa* in his subsequent account, I suspect that it is more or less like *Ilyanassa* and is capable of little or no pedal-wave movement. The locomotion of such gastropods I should designate as due to *arhythmic* pedal movements as contrasted with *rhythmic* pedal movements, such as have been fully classified by Vlès.

It is a significant fact that all gastropods, irrespective of their type of movement (direct or retrograde), are restricted to forward locomotion. None, so far as I am aware, can reverse and move backward as, for instance, an earthworm can. Whatever differences these various types of pedal movements possess, they still lead to but one result, the forward locomotion of the snail.

#### THE GASTROPOD FOOT AS A HOLDFEET

The snail's foot subserves the double function of attachment and locomotion. As means of attachment snails secrete a bed of mucus, and use the foot as a sucker. Both methods are commonly employed by the same species, but in a given form one method is usually developed much in excess of the other. For instance, in *Helix pomatia*, *Limax maximus*, and other allied species, the moist surface of the expanded foot will stick with some tenacity to glass. But if such an animal be allowed to creep its length over a glass surface and thus spread a bed of mucus on which it can rest, it will be found to have multiplied the strength of its attachment many times. The mucus adheres to the glass and the surface of the foot to the mucus very much more power-

fully than the foot alone can adhere to the glass. That this attachment is due chiefly to the adhesive properties of the mucus and not to the sucking action of the foot, is seen from the fact that the attachment can be completely accomplished over a minute hole in a plate of glass. When a snail in such a position is seized and drawn off, air is sucked in through the hole in the glass as the middle of the foot rises, showing that under these extreme circumstances, the foot does act as a sucker, but in the ordinary resting state of the snail no such suction is exerted. All snails with which I am acquainted deposit more or less mucus and though this is sometimes so small in amount that it can be demonstrated only by means of powdered carmine, it serves, I believe, in so far as it is present, as a means of attachment. This production of mucus is highly developed in the pulmonates. Its relation to creeping on the surface-film of water, as exhibited by many fresh-water snails, has long been recognized.

In some snails the foot serves as an organ of attachment chiefly through its power of suction. The general surface of the foot is applied closely to the substrate after which the central portion is lifted thus converting the foot into a sucker. This kind of attachment is well exemplified in *Patella*, *Crepidula*, etc. *Crepidula fornicata* can be made to creep over a surface of glass and can move with ease and security over a minute hole in the glass. If, however, the snail is disturbed by being touched several times when its foot is over the hole, it will actually dislodge itself by endeavoring to suck firmly to the glass, for in so doing it will fill to repletion the forming concavity on the underside of its foot by sucking water through the underlying hole. When one contrasts the difficulty with which *Crepidula* is dislodged from its natural surface of attachment, particularly after it has been induced to exert full suction, with the ease with which it can be made to dislodge itself when over a small hole, the magnitude of its power of suction becomes apparent. The action of the foot of *Aplysia* as a suction apparatus has already been demonstrated by Jordan ('01). These two methods of attachment, suction, and adhesion through mucus are the chief means by which snails hold to the surfaces on which they creep.

## THE GASTROPOD FOOT AS A LOCOMOTOR ORGAN

Locomotion by the gastropod foot, is not dependent upon ciliary action but is a muscular operation as shown by Dubois and Vlès ('07). The precise way in which the movements of locomotion are accomplished can best be made out by examining good examples of direct and retrograde movement. The first is well exemplified in *Helix pomatia* and *Limax maximus*; the second in *Chiton tuberculatus* and *Dolabrifera virens*.

In an expanded and actively creeping *Helix pomatia*, the foot may measure as much as seven to eight centimeters in length by two and a half in width. Over this a succession of transverse, dark-brownish waves run from posterior to anterior. At any instant there may be as many as ten or a dozen such waves on the foot. Each wave is separated from its neighbor by a space equal to about three-times its own thickness. The waves travel over the foot in about thirty seconds, or at a rate of a centimeter in seven to eight seconds. These records, taken from a normal individual, agree fairly well with those given by Bohn ('02) and by Biedermann ('05).

As the snail creeps, it spreads from the mucous gland at the anterior edge of its foot a broad path of slime over which it makes its way. An active snail marks its course in this manner by a long track of slime. A somewhat exhausted snail, when placed upon an appropriate substrate, will almost always creep far enough to lay a mucous path that will subtend the whole of its foot, after which it will cease creeping. If it is removed to another position, it will usually repeat this operation, but it will seldom creep farther. This habit is doubtless connected with the effectual attachment of its foot to the substrate.

Locomotion in *Helix*, like that in other pulmonates (Künkel, '03), is apparently inseparable from the wave movement of its foot. When a snail is placed upon a glass plate preparatory to creeping, it lengthens and expands its foot; almost immediately thereafter pedal waves appear and the animal begins to move forward. Such a snail will creep over a perforation in a glass plate

without sucking air through the perforation, thus demonstrating that its attachment in locomotion, as in rest, is due to adhesion and not to suction. In fact in a creeping *Helix* the foot not only does not suck but actually presses on the substrate. If, as the snail creeps, a bubble of air is introduced under it by a capillary tube or other means, this air will usually escape at the edge of the foot in such a way as to show that it was under considerable pressure. The action of such bubbles demonstrates that the foot as a whole is firmly attached to the mucous substrate, in fact presses against it. Locomotion in *Helix pomatia*, then, has to overcome under ordinary circumstances only the adhesion of the foot and this is accomplished apparently by the pedal waves. In snails in which the attachment is due to suction as well as to adhesion, locomotion requires that both attractive forces shall have been overcome, but, as suction is muscular, it seems likely that this would be relaxed somewhat, as seems to be the case in *Crepidula*, before locomotion begins.

How the pedal waves accomplish locomotion is still a disputed question. According to von Uexküll ('09, p. 181), who has followed Jordan ('01) and Biedermann ('05) in many particulars, each pedal wave is formed by the contraction of the longitudinal muscles of the foot and takes the form of a slight swelling on the underside of the organ. Such a wave, as von Uexküll rightly remarks, would effect nothing by way of locomotion unless some portion of the foot were fixed. Von Uexküll ('09, p. 187) believes that the foot is provided with some such mechanical device as the setae of the earthworm, which, resist backward movement while they allow forward motion and that, therefore, the region in front of each wave may be regarded as a fixed region. Hence the contraction waves would always draw that portion of the foot where they temporarily were forward over the substrate toward the fixed point in front and as a result forward locomotion would be accomplished.

Although this explanation is free from mechanical objections, it is doubtful whether it really applies to the case in hand. Von Uexküll has maintained in support of this view, that a snail can

be slipped over a glass plate more easily forward than backward, just as an earthworm can be drawn over an appropriate surface more easily headward than tailward. I must confess that I have not been able to convince myself that there is any difference in this respect in *Helix pomatia* or *Limax maximus*; both seem to slip over the glass forward and backward with equal ease.

Moreover, the view advanced by von Uexküll is based upon what I believe to be a somewhat erroneous conception of the pedal wave. Biedermann ('05, p. 11) pointed out that the foot of *Helix pomatia* has great advantages over that of many other gastropods for studies of this kind because of the numerous small specks contained in its outer layer. These specks can be discerned clearly by means of a hand lens and they give a true picture of the movements of the foot. As watched through a plate of glass over which the animal is creeping, they can be seen, as Biedermann has described, to move momentarily forward, then come to rest, and then again to move forward. This is best demonstrated on a sheet of glass on which there are numerous scratches. Such scratches serve as landmarks and by them it can be seen that the minute specks in the foot do remain essentially fixed in position and then momentarily move forward to assume again for a brief period a position of rest. When this motion is examined in relation to the foot as a whole, it is evident that the forward motion takes place in the dark waves and that quiescence is characteristic of the intermediate lighter portions of the foot. Each wave, then, is a pulse of forward motion and the rest of the foot is momentarily quiescent. The area covered by the waves is probably a fourth or a fifth of the total area of the foot. At any moment, therefore, about three-fourths to four-fifths of the surface of the foot is stationary and about one-fourth to one-fifth is moving forward. In other words the snail stands on the greater part of its foot while it moves forward with a much lesser part.

Essentially the same conditions as have been described for *Helix pomatia* can be demonstrated in *Limax maximus*. If particles of carmine be driven into the substance of the median, active band of the foot of this slug, they can be seen to exhibit exactly

the same type of movement as has been described for the specks in the foot of *Helix*. In *Limax* the waves, however, are light in color, instead of being dark as in *Helix*, and their surfaces, as seen in the air, are marked with fine wrinkles transverse to the longitudinal axis of the animal. These wrinkles show that the waves are regions of longitudinal contraction, as has been maintained by most recent writers on this subject.

The chief error in most previous accounts of the locomotion of the gastropod foot is found in the physical configuration ascribed to the underside of this organ. Biedermann ('05, pp. 10, 17) states that the waves are convexities on the surface of the foot and that they press more firmly against the substrate than does the rest of the foot. This view was adopted by von Uexküll ('09, p. 187) in his discussion of gastropod locomotion. In *Helix pomatia* it is by no means easy to determine whether the waves are convexities or not, for the reason that they are at most only very slightly different in level from the general surface of the foot. On inspecting by reflected light the free ventral surface of a part of a *Helix* foot over which waves were running, I was unable to tell with certainty whether the surfaces of the waves were convex, concave, or flat. If, however, the creeping foot be closely studied through glass, evidence of a conclusive kind can be found. If, under these circumstances, a very minute air bubble entangled in the mucus under the snail is watched, it will be seen to change its form and position slightly as each wave passes over it. As the wave approaches it, it will elongate slightly on its face next the wave and at times move a little towards the wave, and as the wave leaves it, it will elongate slightly in the opposite direction and at times follow slightly the retreating wave. The motions of the bubble are exactly those that should be expected provided the wave exerted a slight suction in its passage and the reverse of what would occur supposing the wave pressed upon the bubble. The evidence, though slight, is clear and I, therefore, believe that each wave on the underside of the foot of *Helix pomatia* is a slight concavity.

Although the configuration of the surface of the wave in *Helix pomatia* could be determined only indirectly, in *Limax maximus*

it can be seen with distinctness. If the anterior part of the foot of this slug be applied to a glass surface, the pedal waves appear quickly over the whole foot. On inspecting the portion of the foot not yet in contact with the glass, the waves can be identified as dark bands alternating with light areas. On examining from the side the portion of the foot not yet in contact with the glass, it can be clearly seen that the waves are concavities in the foot as compared with the areas between the waves. I am, therefore, entirely convinced that, contrary to the opinion expressed by Biedermann and others, the pedal waves of the gastropods are concavities and not convexities on the foot. In these concavities, which are probably filled with the more fluid portion of the mucus, the foot moves forward, the rest of this organ being temporarily at a standstill.

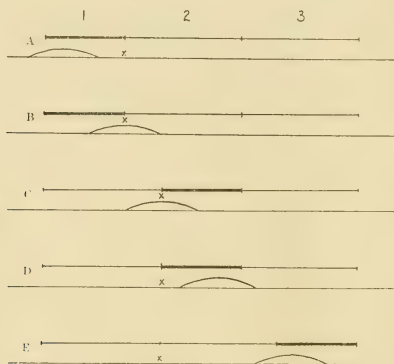
The mechanical advantage of this arrangement must be obvious. The snail is attached to the substrate chiefly by adhesion to the denser mucus. This attractive force is overcome by drawing certain parts of the foot, the region of the waves, away from the substrate. These parts are then in a position to move with reduced resistance and are momentarily shifted forward while the snail supports itself on the rest of its foot. As this release from adhesion is propagated as a wave over the whole of the foot, this whole organ, together with the rest of the snail, is eventually moved forward. At first thought it might seem that such a wave movement could not produce so uniform a motion as snails show, but it must be remembered that the uniformity of this movement is seen only in parts of the animal some distance from the foot. On the foot itself the operation is alternate movement and rest, which becomes more and more continuous motion as points on the body more and more distant from the foot are reached. The locomotion is in many fundamental respects like that of the human being. In our locomotion each foot is alternately at rest and in motion and yet distant parts of our body, like the head, show a motion which in comparison with that of our feet is almost continuously uniform. In fact, a ditaxic gastropod with alternate, direct, single waves on the foot would almost exactly reproduce the method of locomotion found in the human being.

Tectarius, as already noted, practically fulfills these conditions except that its waves are retrograde. This general theory of the mechanics of gastropod locomotion is an elaboration of the views already set forth by Jordan ('01).

It is not my purpose in this paper to enter into an account of the musculature by which the movements already described are carried out, for I have made no observations on this part of the subject. It is, however, pertinent to show that the elements of motion implied in the preceding description are not inconsistent with the general structure of the snail's foot. The work of Jordan ('01), Biedermann ('05), and others shows conclusively, I believe, that the musculature of the snail's foot works against the elastic-walled, fluid-filled cavities of the animal's interior and that these cavities are often temporarily closed from one another. It is these spaces which, acting collectively as a vacuolated, erectile tissue, give rise to such rigidity as is possessed by the expanded foot of the snail. In this tissue two sets of muscles, longitudinal and dorso-ventral, have been identified. The dorso-ventral muscles lift the foot locally from the substrate. They are imbedded in the vacuolated tissue already mentioned and when they contract, their dorsal ends, being more firmly set than their ventral ones, serve as relatively fixed points and the ventral ones, therefore, move. The mechanical support that these muscles receive comes primarily from the tissue adjacent to their dorsal ends which in turn gets its support from other tissues reaching to the parts of the foot fixed on the substrate in front and behind the region of elevation. The action of the ventral end lifts the foot locally and overcomes adhesion in the given region. When the muscle relaxes, the portion of the foot that was elevated is returned to its former level chiefly by the elastic action of the vacuolated tissue and the muscle recovers its original length and position. This action of the dorso-ventral muscles takes place in sequence from behind forward and thus a concave wave runs on the surface of the foot from tail to head.

The second element in the pedal wave is the forward movement of that portion of the foot which is temporarily lifted from the substrate. This must be accomplished by the contraction of the

longitudinal muscles and can be best pictured by reference to the accompanying diagrams. These diagrams represent steps in the passage of a concave wave over the foot of a snail from an anterior position to a posterior one (left to right in the diagram) whereby the point  $x$  is temporarily released from full adhesion to the mucous surface, moved forward, and brought to full adhesion again. The point  $x$  is supposed to be associated with a particular longitudinal muscle fiber, number 2, through whose action it is moved. In *A*, this fiber is shown in its relaxed condition with the wave approaching. In *B*, the wave has released the point  $x$  from full



adhesion. In *C*, fiber 2 has contracted and since the posterior end of it is over a released part of the foot and the anterior end over a fixed part, the posterior end with the underlying point  $x$  has been moved anteriorly. In *D*, the fiber remains contracted and the point  $x$  has come again to adhere to the substrate. In *E*, the wave has reached the next longitudinal fibre anterior, number 3, which has contracted and drawn out the relaxing fiber, number 2, to its original length and position in reference to point  $x$ . The contraction of each longitudinal fibre then serves two purposes: it moves the foot forward as the releasing wave passes over the region and it extends the relaxing posterior fiber. In this way each

point on the foot is lifted, moved forward, and set down again and thus the foot, and with it the animal as a whole moves forward. From this theoretic consideration, it is evident that the theory of pedal-wave action advanced in the preceding paragraphs is entirely consistent with such an arrangement of muscles as has long been known to occur in the gastropod foot.

Vlès ('07) has called attention to the fact that the majority of theories as to the locomotor action of the gastropod foot apply only to the direct type of movement and do not take into account the retrograde type. The theory put forward in this paper is believed to apply equally well to both types. Among retrograde gastropods, *Chiton tuberculatus* is an excellent example. This mollusc uses its foot as a sucker, but nevertheless can creep with considerable rapidity. It exhibits, as a rule, not more than two waves on the foot at a time; these course posteriorly at the rate of about a centimeter in five seconds. In a *Chiton* creeping over a glass plate, the wave when viewed from the side can be seen to be an area lifted well off the substrate. This feature is much more conspicuous in *Chiton* than in any other mollusc that I have examined. As in the pulmonates, the surface of the *Chiton* foot in direct contact with the substrate is motionless; that in the wave area moves forward. At any moment about one quarter of the *Chiton* foot is moving forward while the animal supports itself on the remaining three quarters.

In *Dolabrifera* the foot is pear-shaped in outline with the rounded end posterior. It is about 8 mm. in length. In creeping, one to two waves can be seen on its surface at once; each wave sweeps the length of the foot in about seven seconds. As in *Chiton*, the waves can be clearly seen to be areas in which the foot is lifted completely from the substrate to which the rest of the foot is firmly applied. The pedal surface is mottled and in the wave area it can be seen to be moving forward, whereas on the rest of the foot it is motionless. The total wave area is about one-half the total area of the foot.

The conditions in *Chiton* and in *Dolabrifera* are essentially similar to those in the pulmonates, except that the pedal waves progress posteriorly instead of anteriorly, *i.e.*, the dorso-ventral

muscles contract in sequence from the anterior to the posterior end instead of the reverse and the longitudinal muscles follow the same sequence; otherwise they act as they do in the direct type. It is evident from this brief discussion of the nature of the waves in the retrograde type that the theory developed in connection with the direct type applies perfectly to this second type.

It remains still to point out that what I have called the arhythmic form of pedal locomotion, a form well exemplified in *Ilyanassa*, may be explained on the same general basis as that which has just been given for the two types of arhythmic locomotion. If the foot of such a snail as *Ilyanassa* be thought of as composed of a multitude of small areas, each one of which can be lifted from the substrate, moved forward, and set down again separately, and that this action takes place irregularly and without reference to any sequence, it can easily be seen how the animal could move forward but without the formation of pedal waves. It is my belief that this is the condition in the foot of the arhythmic gastropods, but because of the small size of *Ilyanassa*, I have not been able to subject this opinion to experimental test.

Before closing this paper, I wish to add a word concerning the very remarkable method of locomotion observed by Carlson ('05) in *Helix dupetithouarsi*. The movement carried out by this snail is appropriately described as a gallop, both from its rate and configuration. The snail on strong provocation lifts the head and projects it forward, and eventually brings it to the ground, thus initiating a giant wave which proceeds backward over the length of the body. Several such waves may be present at once. Carlson suggests that this movement is only an exaggerated form of the ordinary locomotion, but I am inclined to agree with Jordan ('05, p. 104) that this is probably an entirely different type of locomotion and I suspect that this snail also possesses the typical pedal wave. In fact it seems to me likely that the gallop was, so to speak, superimposed on the pedal wave system and, had the snail when in gallop been examined from below, the pedal waves would have been seen in operation in conjunction with the body waves. I am the more inclined to the view that the gallop is an independent form of locomotion as compared with the pedal

waves, because in the gallop the body waves of this species, as reported by Carlson, were *retrograde* whereas the pedal waves in all *Helices* thus far reported are *direct*.

#### SUMMARY

Ordinary gastropod locomotion is accomplished either without pedal waves (arhythmic) or with pedal waves (rhythmic). In rhythmic locomotion the waves may run from posterior to anterior (direct) or the reverse (retrograde). The foot may exhibit one (monotaxic), two (ditaxic), or four (tetrataxic) series of waves. In the ditaxic foot the waves may be alternate or opposite.

The gastropod foot is an organ of attachment through adhesion (mucus) or suction, or both.

The pedal wave is an area of the foot that is lifted off the substrate as compared with the rest of the foot and thereby freed more or less from adhesion. It is also the region of the foot that moves forward, the rest of the foot remaining temporarily stationary. Locomotion is the cumulative result of local forward motion on the part of one section of the foot after another till the whole foot has been moved. The same type of muscular movement as that seen in rhythmic locomotion can be present in a diffuse form (not wave-like) in a gastropod foot and will result in locomotion.

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# THE REGULATORY PROCESSES IN ORGANISMS

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Introduction .....	171
The organism as a physico-chemical system .....	173
1. The relation between metabolism and structure.....	173
2. Physiological correlation and the physiological system or individual..	179
3. The basis and nature of physiological correlation.....	181
The nature of regulation .....	182
1. Organic or physiological equilibrium and equilibration.....	182
2. Regulation as equilibration.....	188
The regulatory processes.....	199
1. The relation between form regulation and functional regulation.....	199
2. The inducing conditions and the results.....	199
3. The provisional classification of the regulatory processes.....	200
a. The two methods of regulation.....	200
b. Regulatory compensation .....	202
c. Regulatory transformation.....	205
The nature of reconstitution.....	207
1. Restitution or reconstitution? .....	207
2. The initiating factor in reconstitution.....	211
3. The process of equilibration in reconstitution.....	212
4. The complexity of reconstitution .....	215
5. The limits of reconstitution .....	217
Reproduction in general as a form of reconstitution.....	218
Conclusion .....	221
Bibliography .....	222

## INTRODUCTION

Of late years the term 'regulation' has come into such general use and has been applied to so wide a range of organic phenomena, that it seems desirable to attempt a general consideration and analysis of the regulatory processes from the present viewpoint of physiology. The biologist who takes the position that there is at the present time, when the investigation and analysis of the physics and chemistry of the organic processes is still only

at its beginning, no adequate basis for 'vitalistic' interpretations of regulatory phenomena finds but little satisfaction or enlightenment in Driesch's 'entelechy' or in other assumptions of the neo-vitalistic school.

In the present state of our knowledge these views are and must remain expressions of personal opinion. Driesch's first two "Be-weise der Autonomie der Lebensvorgänge" (Driesch, '01, '03 etc.), which are based on certain phenomena of form regulation, constitute proofs only when we accept Driesch's premises, and as I have pointed out (Child, '08b) these premises are pure assumptions. Neither Driesch nor anyone else has placed them on a foundation of fact. The existence of the 'harmonious-equipotential system,' for example, which is of so great importance to Driesch, is a matter of assumption, not of fact. So far as the systems, which according to Driesch belong in this category, have been thoroughly examined, they have shown themselves to be neither harmonious nor equipotential in Driesch's sense, and to the extent which he has assumed. It is of course easy to assume, as Driesch has done, that the harmony of these systems is due to entelechy and their limitations to physico-chemical factors, but such assumptions, since they are so manifestly invented *ad hoc*, do not carry conviction to the minds of most biologists, whatever, their effect upon their author.

Much the same is true of other modern vitalistic hypotheses: as expressions of personal opinion, they are of great interest in the history of scientific thought, but none of them thus far has presented any convincing arguments in its own support.

Furthermore, with the exception of Driesch's analytical consideration of the regulatory phenomena in organisms, most of the recent published works of general character, which concern themselves primarily with the regulations which involve the visible morphological features of the organism, *e. g.*, the books of Morgan ('07), Korschelt ('07) and Przibram ('09), have been devoted chiefly to the descriptive, rather than the analytical and interpretative aspects of the subject.

In view of these facts, an attempt at physiological analysis of the regulatory processes or of some of them can scarcely be re-

garded as superfluous. The further my own investigations in this field proceed, the more completely I am convinced that those phenomena, which we are accustomed to call regulations are among the most characteristic, perhaps it is not too much to say, the most characteristic phenomena of life.

The views of different authors concerning the relation between regulatory and 'normal' or 'typical' phenomena are very different. Roux ('95, II, pp. 843-4), for example, makes a sharp distinction between typical and regulatory development, though he admits that the distinction is analytical rather than practical. For Driesch regulation is a return approach to the normal condition, after this condition has been disturbed by some external factor. Many physiologists, on the other hand, have used the term 'regulation' in a much broader sense, as applying not only to the extra-normal or extra-typical but at least to many of the most typical phenomena of life.

Because there is no general agreement concerning the real basis and nature of these processes, and because the regulations are of great importance for any interpretation of life, it seems worth while to undertake a brief analysis of them and particularly of the regulations which involve form and structure to a large extent. The present paper is concerned with such an analysis.

## THE ORGANISM AS A PHYSICO-CHEMICAL SYSTEM

### *1. The relation between metabolism and structure*

The structural basis of living organisms consists primarily of colloids. These colloids, together with water, make up the greater portion of what we are accustomed to call protoplasm and in this protoplasm the various reactions and processes which characterize life occur. The universal association of colloids with life suggests that these substances play an important part in some manner in determining some of the characteristic features of life.

Metabolism has been commonly conceived in the past as consisting, on the one hand, of the synthesis of an exceedingly complex

and highly labile molecule, and on the other, of the breaking down of this molecule in functional activity. According to this view the colloid structure built up is used in function and must be continually replaced.

During recent years, however, many facts have been discovered which seem to make necessary some modification of this view of the relation between metabolism and colloid structure. In the first place, most proteids, and indeed most organic colloids, are relatively inactive chemically. The common interpretation of this fact has been that death involved, or perhaps consisted in a change from lability to relative stability of the proteid molecule. But the recent work of Fischer and others upon proteids makes it highly probable that the proteid molecule, although very large, is not so complex as has been supposed, but may be polymeric in high degree. Thus the assumption of extreme lability of this molecule in the living organism becomes even more difficult than before.

Work along other lines has demonstrated that the nitrogen metabolism is only a fraction of the total metabolism of the organism and that it does not necessarily increase in proportion to functional activity. Moreover, the nitrogen requirement for maintenance in animals is apparently much smaller than had been supposed. All of these facts seem to indicate that in the living organism, as *in vitro*, the proteids, or many of them, are relatively inactive chemically; that after they are formed, they are excluded to a large extent from metabolism simply because of their relative inactivity. If these conclusions be correct, the accumulation of proteids in the organism is a process not very different from the deposition of other forms of inactive substance in or about the cell, *e. g.*, chitin, insoluble salts, etc. Indeed it is highly probable that the accumulation of most or all structural substances in the organism is due to the fact that they are relatively inactive under the existing conditions. After they have arisen in metabolism they persist or disappear much more slowly than other substances, simply because under the existing conditions they do not enter chemical reactions as readily as other substances.

But the proteids and other substances deposited in the cell are not absolutely inactive and undoubtedly do enter metabolism

to some extent at all times. Under certain conditions, however, *i. e.*, in the absence of certain other substances, they, or some of them, may reënter metabolism to a much larger extent and furnish energy. It is a familiar fact that in the absence of nutritive material from without the organism uses up its own substance, *i. e.*, the relatively inactive substances which under other conditions had accumulated in it. In certain of the lower organisms this process may continue until the organism is reduced to a minute fraction of its original size. These facts do not, however, conflict with the suggestions made above as to the relative inactivity of structural substance, but serve rather to confirm the idea that the accumulation of these substances, when other nutritive material is present, is due to their relative inactivity.

Various authors have attempted to distinguish between a morphological and a functional metabolism, but it is doubtful whether such a distinction is valid, except as an expression of the fact that substances of different degrees of chemical activity arise in the course of metabolism and that certain of the less active substances constitute the structural basis of the organism, while others undergo chemical transformation and elimination.

It is of course not merely the nature of the substances themselves, but the existing conditions as well, which determine the degree of activity or inactivity. Under certain conditions a cell or an organ may accumulate certain substances and so acquire a certain characteristic structure, while under altered conditions these substances may rapidly disappear and others be accumulated. Thus, for example, the oöcyte, during its growth period, accumulates yolk, which under the existing conditions is almost wholly inactive chemically and so appears as a structure-building substance. But when fertilization occurs the conditions within the cell are so altered that the accumulated yolk rapidly reënters metabolism and serves as nutritive material. In fact we may say that the egg does not produce yolk because it is to develop into a new organism, but that it develops as it does because it has accumulated yolk. In the periodic changes in the cell connected with growth and division there is also abundant evidence for the occurrence of changes of this character.

If we may accept this view of structure in the organism, and all the facts are in its favor, then it is actually very similar in its relation to the energy current to the morphological characteristics of a river system except of course that the latter are mechanically produced. The constructed islands and bars, the depositions of the river, represent those particles or masses which have, under the conditions existing at a given time and place, been left behind by the current. Under certain conditions the river may produce structure of a certain kind at a certain point in its course, while under different conditions this structure may disappear and give place to structure of a different kind.

But the most important fact for present purposes is that in the organisms, as in the river, structure, as soon as it appears, begins to influence the metabolism, the energy current. From this time on the metabolic processes, like the flow of the river, occur in a certain structure and here the mutual interactions begin. Of the character of these interactions in organisms we are only beginning to obtain some vague conceptions, but that they occur, it is impossible to doubt.

Perhaps a few words will not be out of place concerning the bearing of these facts and suggestions upon the theory of 'formative substances' which has played a considerable rôle in embryological investigation during the last few years. Most of the supporters of this theory have attempted to identify the so-called formative substances with visible granules or other accumulations in the cytoplasm, without considering the fact that the appearance of these substances in visible structural form indicates that they are, at least for the time being, relatively inactive, and that they are first of all products or incidents of metabolism (Child, '06b). Of course some or all of these substances might reënter metabolism under altered conditions and so play a part in determining its character, but the important point is that they are indications of a difference in metabolism already existing in the different regions where they are formed. We might expect that the differences in metabolism, which are certainly more important as formative factors than these accumulations of granules, would persist in the different regions, even if the granules could

be removed. Fortunately the embryologists themselves have now a method of removing these granules from their usual position, *i. e.*, the method of centrifuging the egg, and the results of recent experiments along this line indicate, as was to be expected, that the granules are quite unessential to regional localization and differentiation of the embryonic structures.

It is evident from the above suggestions that our fundamental conceptions of the relation between structure and function in organisms must be intimately connected with our ideas concerning the nature of colloid substances and their significance as a substratum or medium for chemical reactions. Within recent years it has been pointed out repeatedly that these substances afford various means for the partial or total isolation of different chemical reactions in organisms and that their mere presence may bring about such isolation, *e. g.*, by the formation of semipermeable membranes. Here then we have a physico-chemical basis for localization and differentiation. Moreover, the changes in the physical aggregate condition of colloids, together with the possibility of the simultaneous existence of different phases of high and low water-content, must play a part in determining the degree and place of dissociation of various substances, and therefore in determining the speed of reactions in different regions, as well as the occurrence or non-occurrence of certain reactions at particular points. It is then, to say the least, highly probable that the possibilities of localization, physiological specification and the accompanying possibilities of physiological correlation of parts and of regulation are very closely connected with the fact that the formation of colloids is a component of the reaction complex known as metabolism. Moreover, as I have attempted to show in another paper (Child, '11b), the accumulation of relatively inactive substances, particularly colloids, in the cell is undoubtedly a factor in senescence, in that it constitutes an obstacle to the metabolic interchange and so brings about a decrease in the rate of metabolism.

If the conclusion be correct, that the visible structural elements of the cell are, at least for the time being, relatively inactive chemically, then it follows that these elements do not represent the

'living substance' in the stricter sense, but are really the least 'alive' of any part. The reactions which furnish the energy of life undoubtedly occur, at least in large measure, in the more fluid parts of the cell, the parts which present the least characteristic structure. The so-called living substance is actually then, so far as it presents a visible structure, chiefly a substratum or medium in which the reactions occur, and is itself the product of past reactions. That these structural elements, as they accumulate, must modify the rate and character of the reactions to an increasing extent, cannot be doubted. The advancing specialization of metabolism in different organs and cells is probably closely connected with the fact that these parts produce different structural elements, either in consequence of an original specification or in consequence of different correlative or external conditions which induce specification.

If we accept this view of the relation between function and structure in the organism, we must give up the idea of a definite 'living' substance in the chemical sense, and the basis of life becomes, not a specific substance, but a series of reactions in a field or medium of a certain complex constitution, which is itself the product of past reactions. We can agree with Driesch ('01, p. 140), as regards the absence of a specific living substance, though we cannot follow him in his further conclusions along this line. The life process has become individualized, not because of entelechy, but because it forms its own field or medium of action, as the river forms its channel, particularly in the later stages of its course, where deposition exceeds erosion. To put the matter briefly, life as we know it consists not in metabolism alone nor in a specific substance or structure alone, but in the physiological correlation of processes in a structural medium or substratum of a certain constitution, which makes possible localization and correlation of processes.

It is then the existing relation between the processes and the structural substratum, the mutual interaction and dependence of both, that forms the basis for the phenomena of regulation or equilibration which occur in the organism.

2. *Physiological correlation and the physiological system or individual*

Organisms in general appear in the form of more or less sharply defined physiological systems or individuals and in the more complex organisms we can distinguish systems or individuals of various kind and degree. What is the basis of this unity?

The experimental investigation of organisms has led those who are not yet ready to accept vitalistic hypotheses to the conclusion that two factors are chiefly involved in the formation of a living system or individual, viz. constitution and physiological correlation. In its grosser aspects the first of these is the morphological, the second the physiological factor. Most of us believe, however, that the morphological features of organisms are essentially visible expressions of dynamic processes past or present and that sooner or later we must interpret constitution in dynamic terms. The factor of physiological correlation in the organism is essentially the problem of physiology, for in the final analysis function is impossible without such correlation.

Wherever in the universe unity can be recognized, there some sort and some degree of correlation must exist, either conceptually or as a datum of nature, between the elements which compose the unit, and vice versa, wherever correlation between conceptual or phenomenal elements is recognized or established, there a unity of some sort and some degree exists. On the other hand, the character of the unity is determined by the nature or constitution, however this may have arisen, of its elements.

There is at present no adequate ground for believing that organisms differ from other phenomena in these respects. We cannot conceive an organic individual without correlation of some sort between the parts which compose it, nor can we conceive it without elements or parts of a certain more or less characteristic constitution.

The development of morphology and its separation from other fields of biology during the latter half of the nineteenth century has led, particularly in the field of zoölogy, to the consideration of the problem of constitution apart from that of correlation. But the

introduction of the experimental method into zoölogy has already demonstrated the limited scope and value of pure morphology for the interpretation of life. In the organism as we find it, the two factors, constitution and correlation are mutually determining. We cannot alter the constitution without altering the correlation of parts, neither can we alter correlation without changing constitution to a greater or less extent. The cases of so-called self-differentiation constitute no real exception to this statement,<sup>1</sup> which has the value and significance of a law of nature. Morphology and physiology are inseparable except analytically and their artificial separation can lead only to the formulation of many pseudo-problems and to uncertain or false conclusions and hypotheses.

In so far as the organism is a physiological, *i. e.*, a physico-chemical individual or unity, in so far must physiological correlation exist between its parts in the form of actual physical and chemical processes, conditions and substances. Until it is proven by the profoundest investigation and the strictest analysis that physiological correlation does not suffice to account for the organic individual, there is no need of turning to the vitalistic hypotheses for an interpretation.

Indeed our knowledge of physiological correlation is in its earliest stages. One need only refer to the work on the conduction of stimuli in plants and through protoplasm in general and to the investigations of recent years on the thyreoid, the adrenals, the reproductive organs, the pancreas, etc., as organs of chemical correlation and to the work on hormones, to become aware of the advances in knowledge along this line within the last few years. At present we are willing to believe, in fact we find it difficult not to believe, that every metabolically active organ in the body is an organ of chemical correlation. And we also know that many

<sup>1</sup> As Roux ('95, p. 822 etc.) has pointed out development depends primarily upon correlation and absolute self-differentiation cannot occur. In cases where parts differentiate in a relatively high degree of independence from each other, we must believe, and in some cases, *e. g.*, the nemertean egg, we know that this condition is preceded by, and is the result of an earlier condition in which the parts are in much closer correlation.

of these correlative factors show a high degree of specificity. Moreover, the chemical factors are by no means the only factors in correlation: mechanical and other physical factors also play a part. And finally, the experimental investigations themselves have demonstrated the importance of physiological correlation in morphogenesis.

In short, there is at present every reason to believe that the existence and continuity in time and space of organic individuality are essentially dependent upon physiological correlation, *i.e.*, upon processes and conditions which are accessible to scientific investigation and analysis.

### 3. *The basis and nature of physiological correlation*

That physiological correlation is in general dependent upon the physical and chemical processes and conditions in the various parts which make up the individual cannot be doubted. These in turn are dependent upon the constitution of the parts, which itself depends in part upon preëxisting correlation and to a greater or less extent upon conditions and processes in the extra-individual environment. At every step in our consideration we recognize the mutual interdependence of constitution and correlation.

But if we consider the organic individual only as it exists at the present time, then we may say that the existing physiological correlation between parts is dependent upon the conditions and processes in the parts, however these may have been brought about.

In general we can recognize at present three main groups of correlative factors: first, mechanical or mass correlation (Roux, '95, II, p. 240), which results merely from the existence of mass without respect to constitution; second, substantial or material correlation, which consists in the actual transference or transportation of substance possessing a certain physical or chemical constitution, *e. g.*, chemical correlation; and third, dynamic correlation, of which the essential feature is the transmission of energy rather than the actual transportation of material over any appreciable distance. None of these forms of correlation can be sharply

separated from the others in the final analysis, but in its extreme forms each type is readily distinguishable.

The physiological correlative effect of a part upon others is then the result of all that that part is and has been in the past, of its physical and chemical constitution, its position, its relation to external factors and of the changes which are occurring in it. It is apparent that there exists in physiological correlation the possibility of an almost infinite variety and specificity. Driesch ('09) has recently maintained that the specificity of the 'Restitutionsreiz' together with the specificity of the reaction to it constitute an 'Individualität der Zuordnung' which is inexplicable on a physico-chemical basis and which therefore constitutes a new and independent 'proof' of the 'Autonomie der Lebensvorgänge.' Comment seems scarcely necessary. One sees here merely an assertion, a jump at conclusions, but no proof, where proof of the most convincing character is absolutely essential. If vitalism can present no more convincing arguments than this its future prospects in science are not bright.

## THE NATURE OF REGULATION

### 1. *Organic or physiological equilibrium and equilibration*

One of the most characteristic features of organisms is, as Roux ('95, I, pp. 145, 154, 392, etc.) has said, their continued existence as individuals, their 'Dauerfähigkeit' amid changing internal and external conditions. On the other hand this 'Dauerfähigkeit' is only relative, not absolute, *i. e.*, it is limited. The organism is constantly changing, and so far as our knowledge goes, never twice the same, yet the continuity of individuality is obvious.

Nevertheless the continuity of the existence of individuality must not be emphasized to the exclusion of the fact that under certain conditions this individuality may disappear, at least in the simpler organisms, and be replaced by other individualities in larger or smaller number. Certain factors concerned in this physiological disintegration will be discussed below, but for the present we are concerned with the individual, the system as we see it

in the organism or part which constitutes a unity distinct to a certain extent from others.

When we investigate the processes in the organism, we find that they are very intimately connected with one another: a change in one conditions changes in others. Moreover, and this is an important point, the physiological specification of different parts is not in most cases absolute. In the highest, most complex forms absolute specification is doubtless approximated more or less closely in certain organs, but in general we find that the processes in different parts of the organism are not fixed in character. The characteristic series of reactions in a part does not represent the only possible series, but rather the particular series determined by a particular complex of conditions. A certain process may occur at one time in a certain part, at another time in others. In short there is more or less possibility of substitution among the different parts.

Let us suppose, for example, that a certain correlative factor  $x$  originates in a certain part. Under certain conditions this factor may influence various other parts,  $a\ b\ c—n$ , of which one,  $a$ , let us say, reacts with greater speed or intensity than others. The reaction of this part may itself produce new correlative factors and so alter conditions in the others that their reactions are changed. But if we suppose that the receptivity of the part  $a$  to the correlative factor  $x$  is decreased, or that the part  $a$  is itself removed or rendered incapable of reaction, then the reactions of  $a\ b—n$  or of some of them are not altered or prevented by the effect of the reaction of  $a$ , and these parts may take the place of  $a$  in the system, though perhaps at first reacting more slowly or less intensely than  $a$ , until their constitution has become altered by repeated or continued reactions.

Through such a series of reactions the individuality of the organism is maintained, or restored, even though it may have lost a part. We see exactly such reactions in various organisms, and we can devise physico-chemical systems which show similar correlation between parts and a similar method of maintenance or restoration of something approaching the preëxisting condition. In the system which we devise such processes are processes of equili-

bration. We find that the system is capable within certain limits of attaining or approaching a condition of equilibrium after a disturbance of a previously existing equilibrium.

And again in the law of mass action and the general principles of chemical equilibrium, together with what we know of katalysis, we have the possibility of accounting for a great variety of processes of equilibration in the organism. Until we have exhausted these and other physico-chemical possibilities and found them inadequate, we have no adequate reason for believing that organic individuality and its maintenance are anything unique.

The fact that physiological correlation exists between different parts of an organism must necessarily determine a certain relation, a certain proportionality in the activities of the different parts. It is this relation, this proportion in activity determined by correlation which constitutes what we call organic or physiological equilibrium in the organism. This equilibrium is dynamic, not static, it is an equilibrium of processes, not of masses and it must be dependent either upon physiological correlation, or upon something else which controls the supply of energy to this or that part in very much the way in which the man in charge controls the workings of a complex machine, *e. g.*, a steam-shovel, turning the steam into this or that cylinder as required for the harmonious working of the whole. Driesch's *entelechy* is comparable to the man in charge of the engine.

But it is not the mere existence of an organic equilibrium which constitutes the real problem; it is the apparent power of adjustment, of equilibration, the harmony of action of the parts, as in the engine, which has been regarded as the strongest argument for vitalism. How, the vitalist asks, is it conceivable that a machine with such capacity of adjustment, of equilibration as the organism, which can even repair itself, can be constructed and continue to exist and work unless there is something comparable to the man in charge concerned in these processes.

As a matter of fact this question is based on a wrong conception of the organism. The organism as we see it, *i. e.*, morphologically, is not the machine whose action constitutes life, but rather simply a part of the products of that machine, which accumulate during

its action and as they accumulate, alter and determine the character of its activity. In other words, as the products are formed they become a part of the machine. Starting with the egg, the organism is not, as Driesch asserts that it must be according to the 'machine theory,' a machine developed *for* function (Driesch, '05, p. 790), but rather a machine developed *by* function. The result at any stage represents morphologically the products of a preëxisting machine and physiologically the action of the machine as altered from the preceding stage by the products of its own activity. Each stage of development is the result of the machine plus the product of the preceding stage. Our experiments have shown that physiological correlation, not predetermined harmony is the basis of development, and that where a predetermined harmony appears to exist it is certainly in some cases, probably in others, the result of an earlier condition of correlation.

On the basis of this conception of the organism it is inconceivable that processes of adjustment of the parts to each other, *i. e.*, processes of equilibration, should not occur, both in development in nature and under experimental conditions. The parts are what they are, not simply because of their original constitution, but because they have been acting in correlation with each other. From the moment the organic machine began to work in the first organism 'adjustment' of the parts to each other began and it has continued ever since. Could we but read it completely, every part is a record, an epitome, more or less complete according to circumstances, of what has been going on, not merely in itself, but in the whole organism. Moreover, in different parts this record is written in different characters, in different languages, according to the constitution of the part.

The distinction which Roux makes between the formative and the functional periods of development (Roux, '95, II, p. 281), is, according to this view, not fundamental in character. The formative period is functional and the functional period is formative. But this distinction is based upon the fact that at a certain more or less sharply defined stage of development the accumulated products of the activity of the machine begin to play a more or less definite rôle in its further physical and chemical activity. The adult

organism is not then to be compared with a machine constructed of certain definite parts, which have been put together in some way, and which, after completed construction, begin to function. It is much more nearly comparable to a river, which molds its banks and bottom, forming here a bar, there an island, here a bay, there a point of land, but still flowing on, though its course, its speed, its depth, the character of the substances which it carries in suspension and in solution all are altered by the structural conditions which it has built up by its own past activity. In such a system a wide range of equilibration exists and we see both the adjustment of function to form and of form to function. The relation between structure and function in the organism is similar in character to the relation between the river as an energetic process and its banks and channel. From the moment that the river began to flow it began to produce structural configurations in its environment, the products of its activity accumulated in certain places and modified its flow, but just so long as the flow continues the process of equilibration goes on.<sup>2</sup>

If we consider merely a certain region of the river with the water containing certain substances in suspension and in solution entering at one end, depositing some of these substances and taking up others as conditions determine in the course of its passage, and finally passing out at the other end bearing certain substances more or less different from those which it brought in, the analogy becomes even more complete. In fact this region of the river, together with its bed, shows a real, though chiefly a mechanical rather than a chemical metabolism.

I believe that this comparison between a river with its channel and the organism is far more than a fanciful analogy. The individual organism is merely a section from that current of energy which constitutes the essence of life, and in the individual we see the mutual correlation and interaction between the current and the conditions under which it finds itself, between the energetic process

<sup>2</sup> Rignano ('07) has referred briefly to this analogy between the river and the organism, using the case of a river equilibrating itself in connection with the piers of a bridge to illustrate the process of equilibration in organisms. See also Delage, *L'Hérédité*, etc., 1903.

and the structural features which its activity has produced. As the banks and the channel are 'adjusted' to the activity of the current, and the current to the morphological characteristics of the banks and bed, so, and in no otherwise are structure and function in the organism, correlated with each other. It is absolutely inconceivable that 'adjustment,' equilibration should not occur. So long as the current flows, equilibration must take place in one way or another.

The organism has often been compared to a flame. Roux particularly has carried out this comparison in detail (Roux, '05, p. 109, *et seq.*). Although this analogy contains much that is valuable and on the chemical side is much closer than that of the river, yet on the other hand the morphological features of the river are more nearly comparable to those of the organism, in their localization, their often complex structure and their modifying effect upon the activity of the current. For these reasons I have chosen the river rather than the flame as a physico-chemical system with which the organism may be compared.

When we take the view of the organism suggested above, I believe that Driesch's first two proofs of the autonomy of vital processes (Driesch, '03, p. 74, etc. Cf. also p. 197 below) appear in their proper light. They apply only to the morphological conception of the organism as a machine constructed for function, *i. e.*, to the banks and channel without the river. In the organism the current is working from the beginning, the organism is functioning in one way or another, and the real machine is the process, the function, plus the existing structure which past processes have produced, just as in the case of the river the real machine is the current plus the banks and channel. The process of development in the organism is comparable, not to the digging of a channel into which, after its completion, the water is turned, but to the formation of a channel with certain characteristics determined by a variety of conditions, by the activity of the current itself. From the moment the current begins to flow, structure and function become mutually interdependent and mutually determining, but there can be no river-structure without the current. Machines like the steam engine, constructed by man and considered without

their motive power, are comparable rather to the dead than to the living organism. They are merely the conditions under which the energy acts, but the living organism consists from the beginning of these conditions plus the energy. Development is not comparable to the construction of such a machine by man, but rather to its action after the steam is turned on. Every steam engine possesses a certain power of equilibration dependent upon its constitution, and the only reason its powers in this direction are so narrowly limited is because the energy current and the structure have not been working together from the beginning.

The only possible basis for a scientific, as opposed to a philosophical vitalistic hypothesis is the proof that the energy of organic life is something essentially different from the energy of the physico-chemical world. When the vitalists shall succeed in proving this or even in making it probable, then their views will be given more general consideration. But even the most extreme among this school at the present day do not attempt such proof. If we admit that the energy of the organism is not different from that in the physico-chemical world, then I believe we are forced to regard the organism as a physico-chemical system, for as I have shown above, physico-chemical systems exist in which the relation between structure and function, between the conditions of action and the energy itself, are of the same character as in the organism itself and give rise to a power of equilibration of the same character.

## *2. Regulation as equilibration*

From what has been said it will be at once apparent that the processes which we commonly call regulatory are processes of equilibration in the organism (Holmes, '04, '07, Child, '06, '08a). They enable the organism to persist and to maintain its individuality under changing conditions, although it cannot be supposed that the condition of dynamic equilibrium is the same for different conditions, and indeed we have evidence that it is not. But within certain limits, and for certain factors, the organism is capable of a greater or less degree of equilibration, when a change in external conditions occurs.

The question at once arises as to whether all processes of equilibration are to be regarded as regulations, or only certain of them. By zoölogists the term 'regulation' has been applied mostly to processes occurring under experimental conditions outside the usual range of conditions in nature and the regulations of form and structure have been the chief, though not the only objects of investigation. Jennings ('06) has used the term with reference to phenomena of behavior which are characteristic features of life and not of abnormal or pathological conditions. Among the physiologists also we find the term often used as referring to various changes in metabolism and reactions of different kinds in response to conditions to which every individual is subjected repeatedly.

If we define regulation as a return or approach to a condition of dynamic equilibrium in a living organism after a previously existing condition has been disturbed by some external factor (Child, '06), we shall include all the above phenomena as well as many others. According to this definition, the simplest reflex as well as the restoration of a missing part is a regulation, the simplest correlative compensation in metabolism, as well as the development of a whole from an isolated blastomere of an egg.

Moreover, when a complex part of an organism undergoes an equilibrating change in reaction in response to altered correlation with another part or other parts, a regulation occurs as truly as when the whole organism responds to some change in conditions outside of it. In short, regulations are equilibrating reactions to changes external to the reacting system, whether this system be a part or a whole of an organism.

And finally, regulation is not limited to the return or approach to the preëxisting condition, but may be an approach to a condition very different from that, *i. e.*, the organism or the part may become something more or less widely different from what it was originally. In every case of regeneration of lost parts some of the cells become something different from what they were before the part was removed, and their change is a reaction to altered conditions and specifically to altered correlation.

But that the regulatory process is always and necessarily of advantage to the organism does not follow from the definition.

So far as it enables the organism to persist, it may be of advantage, and I see no escape from Roux's argument (Roux, '95, I, p. 145, 154, etc.), that systems possessing such reactions will persist longer than others. But not all regulatory processes are of advantage to the organism and many of them, *e. g.*, the so-called axial heteromorphoses, lead to its destruction or its disruption, but they are no less regulations because of this result.

According to Driesch ('01, p. 92), "Regulation ist ein am lebenden Organismus geschender Vorgang oder die Änderung eines solchen Vorgangs, durch welchen oder durch welche eine irgendwie gesetzte Störung seines vorher bestandenen 'normalen' Zustands ganz oder teilweise, direkt oder indirekt, kompensirt und so der 'normale' Zustand oder wenigstens eine Annäherung an ihn wieder herbeigeführt wird."

If we accept this definition, then the processes which do not constitute a return or approach to the previously existing 'normal' condition are not regulations. This normal condition is nothing but the condition which corresponds to a certain complex of external factors or to changes within certain limits. Under changed conditions a new equilibrium, not the old, is established. In short, if we accept such a definition, we not only exclude many processes which are as truly regulatory as any, but we are forced to assume the existence of an entelechy or other similar principle to account for the 'normal' condition and its maintenance.

Regulatory processes are determined in character and direction by the nature of the organism, on the one hand, and the nature and amount of the external change, on the other. Under the given conditions, the organism or part is capable of doing only the one thing; under other conditions, or with a different constitution, the regulation may occur in a different manner and may often lead to a different result. In *Planaria*, for example, the course and result of regulation differ according to the size of the piece, the region of the body from which it is taken, the temperature, the nutritive conditions and other factors. To say that the pieces always produce a whole under all these conditions means but little, for the wholes which they produce are not alike. In plants the character of the external change often plays a very large part in deter-

mining the character of the regulatory processes. In many cases, however, in both plants and animals, the action of the external factor is so indeterminate, or the external conditions are so complex that equilibration may occur in various ways under what seem to be, but are not actually similar conditions. Thus, as Jennings has pointed out, in the regulation of behavior the disturbance, the stimulus, may merely bring about reactions of an indeterminate character, which sooner or later, in one way or another lead to equilibration. Evidently then the relation between the character of the external change and the character of the regulatory process differs very widely in different cases.

The initiating factor in regulation is the external change, the disturbance of the preëxisting condition. This change brings about changes within the organism or the part and these in turn lead to changes in the correlative factors, and so to equilibration or to disruption and death, in case the external change is such that equilibration of the system as a whole is impossible. But so long as the energetic processes of life continue in the system, equilibration of some sort must occur. To return to the analogy of the river, so long as the water flows, equilibration of some sort occurs, whatever the changes and whatever the obstacles. The river may alter its course, it may transform its banks and its channel so that they bear little or no resemblance to those existing before the change, it may divide into a number of streams, each of which pursues its own course, according to the conditions under which it finds itself, and builds up its own structural characteristics. In all cases, however, unless the conditions are such as to stop the flow of the water, equilibration takes place in some manner.

The range of regulatory capacity in the organism represents then merely the range of possibilities within which the flow of the current of energy which constitutes metabolism and which is the essential feature of life, is possible. Within these limits it is absolutely inconceivable that regulation or equilibration should not occur. The nature of the process depends upon the nature of the organism and the conditions which it meets.

Equally inconceivable is the occurrence of regulation as a process of life under conditions which stop the metabolic current.

Organisms which meet such conditions are simply eliminated. The survival and elimination of organisms is determined primarily, not by their morphological characteristics, but by their capacity for regulation of one kind or another under the conditions in which they find themselves. To attempt to understand the course of evolution from morphological characteristics alone can only lead to confusion and failure. Only a knowledge of the nature of the metabolic current in organisms and the possibilities of its equilibration under different conditions can lead to a theory of evolution and heredity which will stand.

For example, the evolution of animals and plants, like every other evolution, is based primarily upon differences in the metabolic processes. These undoubtedly originated as regulations and as soon as they had arisen, gave different possibilities of further regulation: in the course of the realization of these different possibilities in accordance with the conditions of existence, animals and plants with their different morphological characteristics have arisen. In each case the visible structure represents merely a partial record of the realized possibilities. All the structural 'adaptations' in both animals and plants are based upon the processes of equilibration of the energy current and must sooner or later be expressed in terms of this current and its environment. They are not the primary and essential features of the organisms, they give us merely an outline, a diagram of the most characteristic activities of the energy current. As the banks and channel of the river, even after the water has ceased to flow, enable us to gain some conception, though a very incomplete one, of what the river has done in the past, so the structure of the organism is merely a rough sketch of what the current of life has done in the way of deposition, arrangement and removal of materials along its course. Many of the past activities of the current are not distinguishable in the structure because their effects were slight or transitory, or because they have been masked or altered by later activity of a different character. As the river in some process of equilibration, *e. g.*, in a flood, a period of increased energy, may sweep away many of the records of its previous activity, so the

organism, in a period of increased metabolism may remove the structural evidences of past metabolism.

In the present state of our knowledge we should think it absurd to attempt to account for the configuration of the banks and bed of the river without taking into account the action of the current. It would remain a miracle, which we could ascribe to the caprice or other quality of a personal creator, or to some other mysterious natural force. In the same way, when we attempt to interpret the structure of organisms without direct reference at every step to the current of energy of which the structure is evidence, we must necessarily go astray or end in confusion or in the most bizarre hypotheses. We can do as Driesch has done and shift the burden to the shoulders of entelechy, to which we can ascribe such qualities as may please us. Or we can speak of biophores and determinants, pangens, or whatever we please to call them, or we may pin our faith to the visible chromosomes, but these are nothing but creators of a type which appeals to certain minds.

On the other hand, when we take as our starting point the process of metabolism, we are proceeding as the physiographer has learned to proceed in his study of rivers. As we learn how metabolism produces structure we shall be able more and more completely to interpret the nature and the past history of the organism from its structure, but at every step we must return to the process, the current, in order to understand, and we can never hope to understand all through structure, simply because structure is an incomplete record. Life is first of all an energy process, a flowing current. All that is relatively stable, all that persists as visible form and structure, represents merely some past action of the current occurring under certain conditions. Almost sixty years ago Huxley said concerning the cells: "They are no more the producers of the vital phenomena than the shells scattered along the sea-beach are the instruments by which the gravitative force of the moon acts upon the ocean. Like these, the cells mark only where the vital tides have been, and how they have acted."<sup>3</sup> And even yet the truth of these words is not recognized as it should be by biologists.

\* British and Foreign Medico-chirurgical Review, vol. 12, p. 314, Oct. 1853. Cited from Whitman, the inadequacy of the cell-theory, Jour. Morph., vol. 8, 1893.

Only when we take into consideration the motive power and the method of its action under the given conditions, can we hope really to advance in our knowledge of how things come to be as they are in the organism, or to determine and predict what they shall be. Man has attained his present position by acquiring knowledge and control of energy in nature. Can he hope to advance in his insight into the problems and his control of the processes of life in any other way?

According to this point of view, life, like every other continuous energetic process, is essentially a series of equilibrations, of regulations. When regulation shall cease, evolution and life will also cease. The power of regulation in organisms is nothing unique, but is something which they possess in common with all energetic processes in nature, which continue for any appreciable time. In fact, strictly speaking, all energetic processes in nature are equilibrations.

As was suggested above, the range of regulatory capacity in organisms is undoubtedly due in large measure to the fact that the process of metabolism produces certain colloid substances, among which the proteids and lipoids are the most characteristic. With the first proteid synthesis under certain conditions in nature the processes of regulation of the type which we find in organisms began. Perhaps we may say that life began here also. The reaction which was concerned in the first synthesis must of course have preceded the completed synthesis, but as water apart from the channel which it forms for itself in its environment is not a river, so a given chemical reaction, or a series of reactions, apart from the conditions which it produces where it takes place, is not life. We may say if we please that life began as a chemical reaction, but we must recognize the fact that the occurrence of that reaction produced certain characteristic conditions, which played a part in determining the course and character of further reactions: in short, the reaction determined the existence of structure and the mutual interrelations between structure and function: and finally, with the existence of structure of colloid nature, the possibility of regulation of the organic type also appeared, and regulation began.

My purpose in laying special emphasis upon the point that regulation is an essential characteristic of life and that life must cease when regulation ceases, is merely to show that the extreme forms of regulation, which occur under experimental or accidental conditions, are in no way different from the processes of life apart from experimental or accidental interference. The capacity for regulation is not something secondary or something acquired in the course of evolution, but it is as inseparable from life itself as the power of equilibration from the flow of the river. Not only life but the universe is an unceasing series of regulations. Every experimental investigation performed with living organisms is, so far as it does not lead to the death of the organism, an investigation of organic regulation, and death itself is an equilibration, though of another type.

To set the regulations off as a special category of phenomena, occurring only in organisms and of secondary or incidental significance in these, must of necessity lead to conclusions of the same character and value as those which would be reached by one who should attempt to investigate the phenomena of equilibration in the river, without considering the flow or the resistance of its banks and bed. Such a one would doubtless marvel at the wonderful harmony of action displayed by the simultaneous disappearance of a part of the bank and the encroachment of the water upon it, or by the appearance of an island and the division of the river into two channels. He would doubtless call attention to the remarkable fact that both the channel and the river were narrow and deep at some points and broad and shallow at others. He might wonder why stones moved along where the bed was steep and only fine particles where it was nearly horizontal. If he were of an investigating turn of mind, he might throw stones into the river and observe the consequences, or he might dig a ditch and turn part of the water into it. Thus he would observe further remarkable harmonies of action. If he were inclined to look for causes, he would probably conclude that the complex of phenomena was determined and controlled by some mysterious being or principle, which, judging from his own ability to bring about harmony of action between different things in his world, he would

conceive to be more or less like himself, though greater, more perfect and more powerful. Doubtless also he would give it a name.

But when once the idea of the flow of the river as a motive power has entered his mind, his whole attitude toward what he has seen is altered. He sees that it is the current which carries particles away from the bank or stones and mud along the channel. On the other hand he sees that the banks confine the river, that the island, which it has formed divides it, that it accommodates its form to the ditch which he has dug and at the same time begins to change it. He begins to realize that the remarkable harmonies which he has observed are the result, on the one hand, of the flow of the river, *i. e.*, in a further analysis, of the characteristics of water, and on the other, of the nature of its banks and bed. He will also realize in time, that just as long as the flow continues these harmonies of action will continue to occur. Then he may begin to investigate the characteristics of currents and of water in general, and later we find him devising water-wheels, dams, pumps etc., *i. e.*, bringing about the most various harmonies of action between the flow of water and other phenomena.

His conception of what he saw was at first more or less similar to that of the vitalist concerning organisms and all his investigation could only end in speculation, which did not advance his real knowledge. But when he once began to realize the action of the current as an energetic and a constructive process, then he saw that the harmonies of action were only apparent, not real, because he was dealing with mutually dependent phenomena rather than with those which were independent and predetermined.

Driesch, for example has maintained in criticism of some of my own earlier statements, that development is for function (Driesch, '95, p. 790) and the same view is apparent in his repeated comparison of the organism to a machine constructed by man. This is as if our hypothetical man should maintain that because he could dig a ditch and turn water into it, therefore the channel of the river must have been constructed by some 'entelechy,' or other principle for the water, and then the water turned in. And more specifically, Driesch's 'proofs' of the autonomy of vital proc-

esses,<sup>4</sup> which are based on the phenomena of regulation are not proofs at all, because the 'machine' which he has in mind is comparable to the dead, rather than to the living organism, to the river frozen solid, rather than to the river flowing. If we could separate a portion of this frozen river with its channel from the rest it would of course remain what it was, *i.e.*, a part, so long as it remained frozen. But if we divert any sufficient quantity of water from the flowing river it is capable of forming a whole which shows all the essential characteristics of the original river, though not identical with it. In short each flowing river, with its banks and bed is a 'machine' according to Driesch's definition, "eine typische chemisch-physikalische Spezifitätskombination" (Driesch, '01, p. 187), and it may become whole when parts are taken from it or when their relative position is changed; moreover, when it is divided, each part may form a whole essentially similar in its processes and structure to the original whole. The existence of such a 'machine' is therefore a sufficient refutation of these 'proofs' of Driesch's. So long as the current flows such regulatory processes are not only possible but necessary, when the conditions arise. Neither the organism nor the river 'remain whole' when parts are taken from them, but they become new wholes, which under similar conditions, may become more or less like the original whole (Child, '08b), but which under other conditions, may be different.

Driesch's error is two-fold: although his general definition of a 'machine' is sufficiently broad, his argument in the 'proofs' is based only on a certain type of machine, *viz.*, that constructed by man *for* function, a type which is wholly passive during its con-

<sup>4</sup> A brief statement of the first two 'proofs' is as follows:

"Erstens: Eine Maschine bleibt nicht dieselbe, wenn man ihr beliebige Teile nimmt oder ihre Teile beliebig verlagert; deshalb kann das sich auf Basis harmonisch-äquipotentieller Systeme abspielende Formbildungsgeschehen kein maschinelles chemisch-physikalisches Geschehen sein.

"Zweitens: Eine nach den drei Dimensionen typisch spezifisch verschiedene Maschine bleibt nicht ganz, wenn sie geteilt wird, deshalb liegt der Genese äquipotentieller Systeme mit komplexen Potenzen im Bereiche des Formbildungsgeschehen kein maschinelles chemisch-physikalisches Geschehen zu Grunde." Driesch, '03, p. 74.)

struction, rather than on a type in which, as in the organism, the structure at each stage is determined by the function and the structure in the preceding stage. The organism is comparable not to the constructed 'machine' alone but to the machine plus the constructing activity, and since Driesch has confined his argument to the type of machine constructed by man for a definite purpose, he is very naturally and logically led to the assumption of a constructor. His 'proofs' are equivalent to the argument that because a ditch built by man for a particular purpose and possessing a specific structure but containing no water does not remain whole or the same when we take away parts of its banks or bottom, therefore the river, as we see it in nature cannot be a physico-chemical system.

Similarly in his consideration of the organism he has failed to take account of the constructive activity of the continuous flow of energy in a given environment. The organism is, he says, constructed *for* function. His position is identical with that of our hypothetical man who concluded that the channel of the river must have been constructed for the water, and like him, Driesch has given his imagined constructor a name, or rather has adopted an old one for it, viz., *entelechy*. Most of us have concluded from our observations and experiments that the channel of the river is constructed by the activity of the current and we have some rather conclusive evidence upon that point. Before he can hope to see his views accepted, our man must actually prove or make it at least probable that this is not so. The burden of proof lies wholly upon him. And similarly, until Driesch can make it at least probable that the organism is constructed for and not by function, instead of merely assuming this to be the fact, he cannot expect to find wide acceptance for his views. Nowhere in Driesch's work do we find any convincing evidence upon this point: Driesch has simply chosen to assume that it is so. I am of course aware that Driesch regards *entelechy* as in constant connection with physico-chemical factors and as working with these as means. But I see no reason why, if we postulate an *entelechy* for the organism, we should not at least be consistent and postulate another for the river.

## THE REGULATORY PROCESSES

*1. The relation between form regulation and functional regulation*

A distinction between regulations of form and regulations of function has very commonly been made. As might be expected from his conception of the organism, Driesch ('01) has attempted to draw the line very sharply. But if we adopt the point of view suggested above the distinction becomes apparent rather than real. First of all every regulation in organisms is primarily an energetic process and secondly, it occurs in a certain structure and must affect that structure to a greater or less extent. On the other hand, every change in structure must lead to a regulation of function. Structural and functional regulation are in fact inseparable in organisms. If we go further and interpret structure in terms of the constructive energy, we may say that all regulations are essentially functional, *i. e.*, energetic.

It is sufficiently evident from what has been said, that the flow of energy in the organism is essential for regulation, and that the structure must play a part in determining its character. It should be possible, therefore, to interpret the regulatory processes in terms of the energy current, *i. e.*, metabolism, and the preëxisting structure. To refer again to the analogy of the river, both the channel and the current are involved to a greater or less extent in each equilibration in the system. The distinction between form regulation and functional regulation is then in part conventional and connected with the separation of morphology and physiology from each other, and in part a matter of convenience, since some regulatory processes involve the visible structure to a much greater extent than others. As in the classification of other natural phenomena, we separate for convenience of thought or reference a graded series into a number of (in this case two) different classes.

*2. The inducing conditions and the results*

As already noted, the first factor in regulation is a change of some sort in the external conditions affecting the system. In the

case of a part of an organism this change may be a change in physiological correlation resulting from changes in other parts, however produced. This change, external to the system concerned, produces an internal change of some sort in some part or parts, and this in turn alters the physiological correlation between the components of the system affected. So long as life continues, these correlative changes must result in equilibration in one way or another. The processes of equilibration may be very different in different cases: they may bring about sooner or later a return or approximation to the preëxisting condition—according to Driesch, this alone constitutes regulation and only when the preëxisting condition was the 'normal' condition. On the other hand, the correlative changes may result in the establishment of, or approach to a condition of equilibrium more or less widely different from the preëxisting, and I believe that most, if not all regulations which we usually regard as an approach or return to the preëxisting condition actually represent an approach to a new equilibrium, often only slightly different from the old; it seems at least doubtful whether the organism ever really returns to a preëxisting condition in the strict sense.

The new equilibrium may differ quantitatively or qualitatively from the old, or it may even result in the separation of the system into a larger or smaller number of systems, more or less completely isolated from each other. In all these cases, as the rate or character of the metabolic processes are changed, changes in structure as well as in function occur to a greater or less degree. The following suggestions for a classification of the regulatory processes are based primarily upon the metabolic processes concerned.

### 3. *A provisional classification of the regulatory processes*

a. *The two methods of regulation.* It is evident that any really analytical classification which is based upon the conception of regulation suggested above must take account, not merely of the visible features, but of the character of the different energetic processes, since regulation is, according to this view, essentially a complex of energetic processes in a substratum of a certain

constitution. Such a classification must also be available for both the so-called functional and form regulations, since every regulation probably involves both to some extent.

Driesch's classification of the regulations (Driesch, '01, p. 95 *et seq.*) is based upon a conception of regulation so widely different from the one developed in this paper that it does not assist us in distinguishing the processes involved. From Driesch's point of view, the physico-chemical processes in regulation are to a large extent of secondary importance and therefore cannot serve as a basis for classification.

At present, however, we are practically unable to attain the proper basis for classification, since our knowledge of the processes involved is incomplete. Nevertheless we can distinguish with more or less certainty the resemblances and differences between different equilibria, and the following suggestions are based upon the character of the equilibria.

We may distinguish two chief type of regulatory processes, first, quantitative equilibrations or *compensations*, and second, qualitative changes in equilibrium or *transformations*. In compensation the rate or intensity of the processes, their continuation in time or their extension in space are concerned: in the transformations their character as energetic processes, *i. e.*, the nature of the chemical reactions and the physical changes. In the compensation the system remains much like that previously existing, as regards its character and the processes of equilibration are quantitative. In the transformations a new system, qualitatively different from that previously existing, arises as the result of equilibration.

Most regulations, as they occur in nature and experiment, involve both compensations and transformation in various degrees. This is especially true of the regulatory processes which follow the removal of a part. Here some parts of the organism undergo transformation in consequence of altered correlation, while compensatory processes of various kinds are evident, both in the increase in size of the new part and often also in a decrease in old parts.

Moreover our use of these terms will depend upon the particular processes to which we have reference in a given case. The process of compensatory growth, for example, is highly complex in charac-

ter and consists in a variety of both compensatory and transformatory processes, but when an increment in all of these processes occurs, the change is quantitative in character and when it constitutes a process or part of a process of equilibration we are justified in calling it a compensation.

It is of course evident that a classification of regulatory processes must finally become identical with the classification of processes occurring in the organism, for, as I have pointed out above, the regulations are not a peculiar form of organic activity; they represent merely the equilibrations resulting from the existence of physiological correlation between parts. But we shall probably always have occasion to refer to the organism, the system, as a whole undergoing equilibration or, relatively speaking, in equilibrium, consequently some means of distinguishing between the different methods of equilibration is useful. This is the chief significance which any classification of the regulations can possess.

*b. Regulatory compensation.* Several different types of compensation can be distinguished, though they do not of course in most cases exist in nature or even in experiment apart from other processes. The following divisions under this head are suggested:

*Incremental compensation:* The system shows an increment as compared with that previously existing.

*Decremental compensation:* the system shows a decrement as compared with that previously existing.

*Reversional compensation:* an increment or a decrement in some part of the system, induced by some external factor is correlatively more or less completely eliminated and the system approaches its previous condition.

*Alterative compensation:* an increment or decrement in one part produces change in the opposite direction in another or in others, so that the proportional relations in the system differ from those previously existing.

The first step in all compensations is of course a change in some part (*a*) induced by some factor external to the system. What particular form of compensation shall occur depends upon the degree of the change in the part and upon the character of the correlation existing between it and other parts (*b, c, d-n*). If for

example the part *a* dominates the other parts, or if the change in *a* is so great that the correlative factors resulting from it become dominant, then an increment in *a* may bring about an incremental compensation, a decrement in *a* a decremental compensation. On the other hand, if the parts *b*, *c*, *d*—*n*, or certain of them, dominate *a*, then they may inhibit or reverse the incremental or decremental change in *a* and reversional compensation results. And finally, alterative compensations occur whenever changes in one part induce correlatively changes in the opposite direction in others.

An incremental compensation occurs when increased metabolism and growth follow the ingestion of food, a decremental compensation, when decreased activity of a sense organ or a muscle induces a correlative decrease in activity and perhaps atrophy in parts with which it is connected, *e. g.*, the center in the case of the sense organ, the tendon, or even the bone in the case of a muscle. In various temperature regulations in warm-blooded animals we have reversional compensations, and finally, in many cases of regeneration and probably also often in normal development, alterative compensations occur, *e. g.*, when increased growth of one part retards correlatively the growth of another or perhaps induces reduction in it.

The chemical substances which arise in the course of metabolism in certain parts very often produce compensations of various kinds in other parts. A good example is the correlative effect of increase in the carbon dioxide in the blood through the nervous system upon the rate of respiration. The recent work of Bayliss and Starling and others on 'hormones' gives us some insight into various other cases of compensation and other regulatory reactions; the distribution of nutritive substances in the starving animal and under various other conditions also constitutes compensations of various kinds; the correlative changes in so-called functional structure are in many cases very characteristic compensations.

Incidentally it may be pointed out that the view of the relation between metabolism and structure suggested above affords a basis for interpretation to a certain extent of the processes of functional hypertrophy and atrophy from disuse.

We have seen that structure-formation of some sort, *i. e.*, the accumulation of relatively inactive substances in or about the cell is a characteristic feature of metabolism. The close relation between the syntheses and the oxidation processes has been pointed out repeatedly by Loeb as well as by others. The structural substances, when once formed, play only a relatively small part in further metabolism, provided other more active substances, *i. e.*, nutritive materials, are at hand, and provided the general character of metabolism is not changed. Any condition, *e. g.*, the 'functional stimulus' which leads to increased metabolic activity of the particular kind which constitutes what we call the special function of the cell or part leads, when nutritive material is present, to increased accumulation of the inactive substances and hypertrophy is the result. On the other hand, in the absence of the functional stimulus, or when its frequency or intensity is decreased, the use of nutritive material and the accumulation of structural substance do not occur or are less rapid, and the result is that below a certain level of functional activity the gradual breaking down of the accumulated substance, which is not immediately connected with the special functional activity of the part, exceeds the constructive processes and decrease in size and atrophy occur. The constructive processes continue only, or very largely, in connection with the functional stimulus and, for the addition of new structure nutritive material must be taken in from without, but this functional activity does not under these conditions, increase proportionally the rate of reëtrance of the structural substances into metabolism; in fact, if other more active substances are present in sufficient quantity, the structural substances may be spared to a large extent.

Hypertrophy and atrophy are then the result of two different kinds of processes, the one connected with the specialized function of the part in its relation to other parts, the other to a considerable degree independent of this except in starving animals. In its 'functional activity' the part builds structure, but does not destroy it to so great an extent. The destructive process is largely independent of function and goes on more or less continuously. Whether hypertrophy or atrophy shall occur in a given case depends merely

on whether the one or the other of these processes is the more rapid. Hypertrophy is then in no sense a 'regeneration in excess'; it is merely a direct result of increased metabolic reactions of the kind which constitute or accompany the 'function' of the part concerned. Nevertheless, it is without doubt a compensation. The occurrence of one series of metabolic reactions determines the occurrence of another series according to chemical laws; the one series furnishes energy, the other forms relatively inactive substances, which persist as structure. Closely related to functional hypertrophy is the growth in size of regenerating parts after their formation: here the 'functional stimulus' is the quantitative factor in the correlative influences from other parts. This factor induces a certain rate or frequency of reaction in the small new part, which leads to rapid accumulation of material, *i. e.*, to hypertrophy (Child, '06a, p. 407). But as the structural substance accumulates, the structure itself constitutes an obstacle to metabolism (Child, '11b) the rate of hypertrophy decreases and finally equilibrium is attained.

*c. Regulatory transformation.* The character of many metabolic reactions is more or less definitely known, but the exact relation of the reactions to the production of a particular kind of visible structure is a much more difficult matter to determine. The visible characteristics of organic structure are by no means adequate criteria of the character of the processes involved in its formation. We are not always justified in concluding from the differences in the visible appearance of structures that the processes concerned in their formation are actually different in nature. Great differences in appearance may arise in the same colloid substance in consequence of differences in aggregate condition or phase. But when we find substances of different constitution in different cells or parts, it is evident that processes of different character were concerned in their formation. Consequently we can often determine that a transformation has occurred by the change in the character of the structural substance. Many features of correlative differentiation, whether in ontogeny in nature or under experimental conditions are undoubtedly transformations, *e. g.*, the formation of a bud from a differentiated cell in

the plant, in consequence of the removal of other vegetative tips, the formation of a hydranth from cells of the stem of *Tubularia*, etc.

The difficulty lies in distinguishing qualitative from quantitative regulations. In living organisms the two are evidently very closely associated, and probably in every regulation which we can observe directly both are concerned. And in the final analysis the question of the relation between quality and quantity in general is involved, though this is scarcely a biological problem.

As regards the further classification of the regulatory transformations, I think that at present the most satisfactory basis for classification is the comparison of the new system with that existing before regulation. The following division of this group of regulations is therefore suggested:

*Progressive transformation*: the regulatory formation of a system possessing a greater degree of complexity, more varied localization of structure and function and consequently more varied correlation than the system existing before regulation.

*Regressive transformation*: the regulatory formation of a system of simpler character than the preëxisting.

*Transgressive transformation*: the regulatory formation of a system which cannot be distinguished as more or less complex, but merely as different from the preëxisting.

Such a classification serves merely to suggest the various possibilities. As our knowledge of the processes concerned in the changes of the organic system increases, the basis of classification will change. Without doubt many progressive transformations occur in normal development. The adult organism is certainly a more complex and qualitatively different system from the blastula, and we know that correlative factors have been concerned in the changes in many parts. A regressive transformation occurs when a part undergoes dedifferentiation in consequence of altered correlation, as in various cases where cells which give rise to new parts first lose their old differentiation.

The group of transgressive transformations possesses little more than a conventional significance, since it is based upon difference from the normal which is essentially merely the usual

Very probably various 'sports' and mutations can be placed under this head, perhaps also certain of the neoplasms, more specifically the malignant tumors, though this is by no means certain.

But whatever the categories which we may establish for the different regulatory processes, the important point is that we should at least make the attempt to find a physico-chemical basis for our analysis. If we do this we cannot separate structure and function since both are merely different aspects of the same process-complex and are dependent upon and determine each other. Doubtless we shall still find it convenient to speak of form regulation as distinguished from functional regulation, but we must remember that the distinction is not a real one and that every regulation in the organism is undoubtedly a regulation of both form and function, of both structure and reactions. Furthermore, we must regard our experiments on regulation as means of analyzing the factors of the process. With the proper care in experiment we can do much toward determining the nature and action of various correlative factors in regulation, and every step in this direction is a step in advance in our knowledge of the system which constitutes the organism.

## THE NATURE OF RECONSTITUTION \*

### *1. Restitution or reconstitution?*

When an organism 'restores' a missing part or in general when a part of an organism forms a whole, the process seems at first glance to be so obviously a restoration in which something removed is replaced, that the term 'restitution' has found very general favor. Although I have used this term to a large extent, it has always seemed inadequate, for the reason that the process is not simply one of restoration but something more. There is no case of so-called restitution known in which the changes following the removal of a part are limited to the formation of a new similar part. In every case changes of one kind or another, quantitative or qualitative, or both, occur in other parts, sometimes limited chiefly to parts adjoining the part removed, sometimes extending throughout the system. The removal of a part

brings about not simply its restitution, but an equilibration extending more or less widely, and strictly speaking, probably throughout the system. The system reconstitutes itself and the whole formed is different from the original quantitatively or qualitatively. The whole process is a complex of equilibrations, of compensations and transformations, resulting in that which we call a whole, but no two wholes are alike.

If for example, we consider this process in a piece of the Planarian body, we find that it differs in rate and character according to size of the piece, region of the body from which it is taken, temperature and other factors which influence metabolism. The animal formed as the result resembles the original in its general shape and activity, but it is far from being identical with it. It is usually smaller than the original, the pharynx may be in quite different position in the body, and the arrangement, number and form of the intestinal branches differs more or less widely, according to conditions. Moreover, under various conditions, various degrees and kinds of incompleteness appear. Some pieces develop only a single eye, or the eyes are partially fused or otherwise different from those in the original animal, some pieces develop no pharynx and no posterior end, others no head or an 'imperfect' one, some develop the postpharyngeal intestinal branches more rapidly and more completely, others the prepharyngeal branches, some produce a larger, others a smaller head, some show more 'regeneration,' others more 'redifferentiation' and so on. If we place the different sorts of wholes under closely similar conditions and give them food they become more or less like each other because these conditions bring about further regulations but these regulations do not properly belong to the regulatory process which resulted from the isolation of the part, but are independent of it and are such as were occurring in the original animal during its life. It is probably not too much to say that no two pieces of the Planarian body attain the same condition in the process of regulation. When we say that because some or all of them produce wholes they are all potentially alike, we are simply assuming that all wholes must be alike, which is obviously untrue. As each piece is different at the start from the others, so it attains a different

result. Driesch's assumption of 'equipotentiality' of different parts is shown by the facts themselves to be incorrect, and I believe that his 'harmonious-equipotential systems' do not exist in nature, as systems capable of development but only as abstractions of the human mind.

For these reasons the term 'restitution' seems to me to carry with it implications which, when we analyze them, we cannot, in the light of the facts, accept. The piece does most certainly not restore what it lost: it reconstitutes itself into something more or less widely different from that of which it formed a part, and this something often possesses visible structural characteristics which we have come to regard as characteristic of a whole. Seeing these resemblances, we abstract from the differences and say that it is the same as that of which it formed a part.

Closer examination shows us that even visibly it is not the same, but different; moreover, visible characteristics are not the sole criterion of resemblance and difference in organisms. The processes occurring are just as characteristic as the visible structure. I have shown elsewhere (Child, '11b) that in *Planaria* the process of form regulation results in a rejuvenation, the pieces after undergoing regulation are physiologically younger than the animals from which they were taken, and the degree of rejuvenation varies with the degree of reconstitucional change. Manifestly then these pieces are not the same after regulation as the wholes of which they formed parts. To say that they are is simply to deny the facts as they stand before us.

In many cases, moreover, the pieces do not produce anything that can be called a whole. Pieces of *Planaria* may produce double heads or double tails, tailless heads or headless forms. For those who with Driesch regard the formation of a 'whole' as the uniform result of so-called restitution, these cases are difficult to interpret. The process of regulation has apparently followed the wrong track, it has gone astray and so failed of the correct result. But when we take the position that the part, when isolated, undergoes a reconstitution, which differs in its results according to the existing conditions, internal and external, we see that these 'abnormalities' differ from 'normal' results simply because different

conditions existed at the beginning or elsewhere in the course of the regulation, and in many cases we can determine what those conditions are. For example, pieces of *Planaria* which give rise to 'wholes' at a certain temperature and under certain other conditions, can be made to produce headless forms or tailless heads, according to the region of the body from which they are taken, by subjecting them to lower temperatures, by starving the animals before beginning the experiment, by placing the pieces in dilute alcohol or ether, etc. Nothing has gone astray in these cases, there is no error, the same laws have been followed as when 'wholes' are produced, different conditions simply lead to different results. Elsewhere (Child, '10c) I have attempted to analyze some of the conditions which bring about so-called heteromorphosis in *Tubularia* and other forms, and have shown that they are similar in character to those which bring about asexual reproduction in nature.

There are many cases in which the occurrence of reconstitution as opposed to restitution is so obvious that there can be no questioning it. A piece from the body of *Hydra*, for example, does not restore the missing parts, but reconstitutes itself into an organism, smaller, simpler, possessing fewer tentacles and undoubtedly physiologically younger than the original animal. In *Clavellina* also, as Driesch himself has shown (Driesch, '02), the isolated branchial region or a part of it does not replace the missing parts, but undergoes a process of reconstitution. In these cases the physiological effect upon the parts remaining of the removal of certain parts is so great that these parts do not retain their original structure, and a dedifferentiation and redifferentiation occurs. But the effects so apparent in these cases are simply more extreme than in cases where only a small part is removed. Przibram ('07) has called attention to a number of cases which show very clearly that the removal of a part results, not in the restoration of the original, but in the establishment of a new equilibrium, differing more or less widely from that.

It is obvious that the process of reconstitution is an equilibration and just as obvious that it leads to different results under different internal and external conditions. As different rivers differ

from each other, so may the results of reconstitution, even in different pieces of the same individual, differ from each other. Moreover, as a certain amount of water does not, except under certain conditions, form a river, so does a piece of an organism reconstitute itself to a whole only under certain conditions.

## 2. *The initiating factor in reconstitution*

As Driesch has pointed out (Driesch, '01), it is evident that reconstitution occurs as the result of the absence ('Nichtmehr-vorhandensein') of something. What is this something? Is it the structure, the form, or is it activity? In plants it is possible to bring about reconstitution, *i. e.*, the formation of new buds, roots, etc., by inhibiting the metabolic activity of the existing growing regions without their removal, *e. g.*, by enclosing them in plaster or in an atmosphere of hydrogen, or even by applying anesthetics locally between them and the regions concerned. In another paper I have considered numerous cases of this sort and have discussed their significance at length (Child, '11a). These facts show very clearly that it is not the form or structure which is involved but a process, an activity, whose effect is transmitted in some way from one part to another. If we stop the metabolism of the one part for a time the effect on the other is the same as if the first part had been removed. These facts alone should be sufficient to prevent us from regarding form regulation as a process distinct from functional regulation. It is the absence of the effect of certain processes in a certain part or in certain parts, which initiates reconstitution. In short, it is the absence or decrease below a certain point of certain physiological correlative factors which were previously present, that initiates reconstitution. In the absence or decreased effectiveness of these correlative factors, the remaining parts, still being subjected to other correlative factors, which may themselves gradually change in consequence of the removal or decreased activity of the part, react in a manner different from their previous reactions, simply because they are under different physiological conditions. Reconstitution is then initiated by a change in physiological correlation. Recently Driesch ('09) has

expressed himself in somewhat similar terms, but he finds nevertheless, as already noted above, that the 'Individualität der Zuordnung' between agent and effect cannot be accounted for on a physico-chemical basis and therefore regards it as a new 'proof' of the autonomy of vital processes.

### 3. *The process of equilibration in reconstitution*

The process of equilibration in reconstitution differs in many details in different cases, but it possesses certain more or less characteristic features, and it is desired to call attention briefly to some of these. The change in physiological correlation is the internal factor which has disturbed the preëxisting condition, whatever that may have been. This change may or may not lead to equilibration of the living organism. If the change be great, if the other parts possess but little capacity for altering their reactions, it may lead to death. On the other hand, it may lead to reconstitution in various ways according to conditions.

Let us consider first the case where a part is removed and is formed again without any great changes in other parts, *e. g.*, the 'regeneration' of the posterior end of *Planaria*.

In the absence of the correlative factors which originated in the part removed (*a*), certain regions (*b*) of the remaining parts (*b, c d --- n*), which were before prevented by these correlative factors from reacting as their own constitution and the correlative factors from other parts would determine, now begin to react in this manner. In the region adjoining the part removed, *i. e.*, in the cells *b*, the correlative factors originating in the parts *cd --- n* are more or less similar in their effect to those which affected the part removed (*a*). So far as they are and remain similar, and so far as the constitution of *b* permits, this region will be forced by the correlative factors to react more or less in the manner of *a*, which is no longer present, and *b* will replace *a* more or less completely and more or less rapidly, according to conditions in the particular case<sup>5</sup>. If

<sup>5</sup> The formation of a new head in *Planaria* or a new hydranth in *Tubularia* is a somewhat different process from the formation of the proximal or posterior end. In these forms the anterior or distal region is physiologically dominant over parts

the cells of the region *b* adjoining the part removed, be chiefly affected correlatively by the removal of *a* or if they react much more rapidly than others further away, the process of formation of *a* will be a 'regeneration.' But if parts further away from *a* are also affected to a considerable extent by the change and are capable of reacting as rapidly or almost as rapidly as *b*, then they may also take part in the process of replacing *a*, which then takes on more or less completely the character of a 'redifferentiation.' In some cases we can determine experimentally whether a part shall be formed chiefly by regeneration or redifferentiation. In *Planaria*, for example, the amount of regeneration, as opposed to redifferentiation, in the formation of a new posterior end increases with increasing distance of the cut surface from the old posterior end. The farther the level of the cut from the old posterior end, the more completely is the development of the new part confined to cells near the cut, and *vice versa*. The cells near the cut are those which are most affected by the removal of the part *a*. Even when this part does not develop anew, they react by healing the wound. That is to say, they change their reactions most rapidly of all cells, they lose their old specification, they become capable of a more rapid metabolism (Child, '11*b*) and being subjected to the correlative factors of the parts *c d---n*, they begin to develop into something more or less like *a* in advance of other parts. The processes in these cells establish certain correlative factors which determine that the cells farther away from the cut shall remain as they are or take other forms of reaction.

But if we decrease the rate of metabolism in *Planaria* by extreme starvation or by the use of anesthetics, then parts which under the

posterior or proximal to it and controls their development directly or indirectly. Briefly stated, the regulatory formation of a dominant part is a reconstitution resulting primarily from isolation, while for the formation of a subordinate part correlation with other parts of the original system is necessary. For example, a piece of the tubularian stem may reconstitute itself into a hydranth without any other parts (Child, '07*a*, *b*, *c*), or a piece of *Planaria* into a head without other parts but a tubularian stem or stolon or a planarian tail is never formed except in connection with a more distal or anterior region of the original organism. The question of the dominance and subordination of parts and its significance will be discussed more fully elsewhere.

usual conditions are formed chiefly by regeneration, *e. g.*, the head, may be formed largely by redifferentiation (Child, '10a). This means simply that the cells near the cut do not react so rapidly under the usual conditions, so that other cells further away have time to change their reactions and take part in the process, while ordinarily they would be prevented from doing this by the correlative factors arising from the activity in the cells near the cut. These instances are merely special cases under the general rule that the less rapidly the missing part is replaced, the more extensive are the changes in the remaining parts, so far as their constitution permits change.

Regeneration and redifferentiation in their extreme forms represent the extreme terms of a graded series, of which all terms are essentially of the same physiological character, *i. e.*, all consist in a change in reaction in consequence of a change in physiological correlation. The designations 'regeneration' and 'redifferentiation' serve merely as convenient descriptions of the visible phenomena.

Where all the cells of the remaining parts are so sensitive to the absence of the correlative factors originating in the part removed that they cannot maintain themselves after its removal, the old structure of all the remaining parts may disappear to a greater or less extent, *i. e.*, a 'dedifferentiation' occurs, as, for example in the isolated pieces of the branchial region of *Clavellina*. In the different cells of the mass the metabolic processes become less specified and in this respect it approaches the 'embryonic' condition, and the correlative factors in the mass approach those existing in the embryo. But during this process some of the cells have been subjected to correlative factors more or less similar to those to which the part removed was subjected at some stage of development, consequently these become in some degree the physiological representatives of that part. In short the system becomes physiologically a whole, but in consequence of the rapid dedifferentiation of the old parts it corresponds to a whole in a relatively early stage of development. From this condition renewed differentiation as a whole results necessarily from continued metabolism,

*i. e.*, continued life. The new whole is, however, different from the old in size, physiological conditions, number and proportion of various parts, etc.

In the process of regeneration in the stricter sense the new part is usually at first small and increases rapidly in size. I believe that this growth in size is essentially similar to the functional hypertrophy of organs. The part which was removed possesses a certain size in relation to other parts, because its size was determined chiefly by correlative factors. Just so far as the new developing part is subjected to similar correlative factors, it will tend to attain the same size as the part removed. Consequently it does not always attain the same size with respect to other parts. In *Planaria* the relative size of the new head differs according to the region of the body from which the piece is taken, to the nutritive condition and various other factors. The process of reconstitution ceases when a certain stage, differing under different conditions, is attained. This stage represents an equilibrium of physiological correlation, *i. e.*, of interaction between the parts; it is primarily a dynamic equilibrium, a proportionality of processes, not of form. We can alter this condition of equilibrium experimentally by food, by starvation, by temperature, and in short by all factors which affect the processes.

In various papers Driesch has distinguished a number of different forms of reconstitution (restitution). His distinctions are based primarily upon differences in the visible phenomena of development or dedifferentiation and for him the chief interest lies in the recognition of the different forms, rather than in the attempt to determine how they differ from each other physiologically, since from his point of view the physiological factors are in many cases only 'means' which the *entelechy* employs. It is impossible to consider here these various forms of reconstitution, and since my point of view is so widely different from that of Driesch such a consideration would show merely that his basis of distinction could not be accepted for purposes of a physiological analysis. While it is convenient to distinguish different forms or methods of reconstitution, I believe that it is much more important to resolve the phenomena into processes.

#### 4. *The complexity of reconstitution*

The process of reconstitution is not a simple process which cannot be analyzed, but rather an exceedingly complex one; in fact its complexity is of the same order and character as that of development. It consists of a series of compensations and transformations in different parts of the system. It is only when we take into account the complexity of physiological correlation between parts and the almost infinite possibility of change and variety in this correlation that we have any hope of gaining an insight into the complex series of events. The specificity of correlation and reaction does not, as Driesch apparently believes (Driesch, '09) constitute a physico-chemically insoluble problem except when we follow Driesch in ignoring the energy current as an equilibrating factor in the organism and as the efficient factor in construction of the visible and tangible characteristics. The energy current performs its work under specific conditions in each case and leads to a specific result. As soon as a specific condition arises in any part of the system, from whatever cause, it determines other specific conditions in at least certain other parts. From the experiments on *Planaria* it is perfectly apparent that the cells at every level of the body posterior to the ganglia, are capable under certain conditions of developing into a head, but under the usual conditions they are prevented from doing this because the correlative factors arising from the presence, *i. e.*, the activities, of a head determine their activities in another direction. As soon as the old head is eliminated from the system, those cells, which in consequence of their past correlation are most similar to it, begin at once to form a new head, provided the piece is not too small and as soon as this occurs it determines correlatively a variety of reactions in other cells. The same may be said of the reconstitution of any part. The place where a particular part shall arise is determined by constitutional and correlative factors in the existing system—so far of course as external factors are not concerned—and as soon as one such place is determined, it determines others and so on. Thus any case of reconstitution consists of a series of regulations, each of which determines others. This

is shown very clearly by the fact that isolation of a part from certain others during the course of reconstitution may alter the course of the process in the part, according to the degree and character of the isolation, *i. e.*, according to the correlative factors which are eliminated. By means of experiments of this kind it is possible even now to determine the action of various correlative factors in different stages of the process of reconstitution.

### 5. *The limits of reconstitution*

In every case the reconstititional processes are limited and determined by existing conditions as the river is limited and defined by its banks and channel which its own activity has constructed in the environment through which it flows. It is not true without qualification that any part of certain organisms is capable of giving rise to any part. Driesch's often repeated statement to this effect requires modification and limitation. The part is at most only provisionally capable of giving rise to any part; in other words, only when it constitutes a component of a system possessing certain characteristics, *i. e.*, only when it is subjected to or isolated from certain correlative factors. This is apparent from every recorded series of observations on reconstitution, except perhaps the most superficial. In some cases the system may consist of a single cell, in others of a large number of cells, but the fact remains the same. The power of reconstitution is limited, not unlimited. As I hope to show elsewhere for *Planaria*, and as I have shown for *Tubularia* (Child, '07a, '07b, '07c), the investigation of these limitations is of the greatest importance in throwing light upon the nature of the reconstititional processes. When we find that the removal of a certain part, or even a certain amount of material, determines a different result from the removal of another part or a larger or smaller amount of material, we are forced to the conclusion that the part or the material removed has some connection with the character, place or other factors in the result, and furthermore, when we find that inhibition of the metabolic processes or certain of them in the part or the material is as effective in certain

respects as the removal of the part or the material, we have attained a basis for investigation and analysis which is proof against such assumptions as those which Driesch has made, *e. g.*, concerning the nature of the 'harmonious-equipotential system.' For Driesch the limitations of the reconstitutive processes appear to be of secondary importance, but I believe that any one who will investigate and analyze these limitations at all thoroughly will find that they are not only essential features of the regulatory processes, but that they afford us one of the best means of gaining some insight into their nature. As water does not constitute a river, except under certain limiting conditions, so certain substances or processes do not constitute an organism or even life except under certain limitations. The water contains the potentialities for giving rise to any kind of a river, as well as other specific 'machines,' but none of these exist until the specific limitations are present. The case is essentially similar as regards the organism. The specific 'machine' exists only so far as the limitations exist. And the investigation of the limitations of reconstitution affords at present one of the best methods, if not the best, for demonstrating this to be a fact.

#### REPRODUCTION IN GENERAL AS A FORM OF RECONSTITUTION

In another paper (Child, '11a) I have discussed at length the significance of physiological isolation of parts as a factor in reproduction. I have shown that certain degrees and kinds of physiological isolation of parts may arise as the result, first, of an increase in size; second, of decrease in correlative control or physiological dominance of a part in consequence of decreased activity in it; third, of decreased conductivity or transmissibility of correlative processes, agents or conditions; fourth, of decreased receptivity, sensitiveness or irritability of certain parts to the correlative factors originating in other parts. Furthermore, we know from experiment, as I have shown, that in a considerable number of cases physiological isolation of parts serves as well as physical isolation by section to bring about reconstitution; and if it were possible to perform the experiment, it is practically certain that we should find the same to be true for many other cases.

In the paper just referred to I have also attempted to show that at least a great variety of natural and experimental forms of reproduction, reduplication of parts, etc. are essentially processes of reconstitution following physiological isolation of parts. The chief difference between them and the cases of reconstitution following experimental section is, first, that the isolation of the part or parts is brought about within the organism physiologically and not by the crude method of cutting the organism into pieces; and second, that this isolation is usually partial at first and differs in degree and kind in different cases.

And finally, I have called attention to certain evidence in support of the view that the formation of sex cells and the development of organisms from them are processes not fundamentally different from other forms of reproduction, *i. e.*, that the sex cells are first physiologically parts of the organism like other organs, and that the development of a new organism from them is initiated by changes similar in character to those which occur in other parts capable of reconstitution, when they are physiologically or physically isolated (Child, '10b, '10c).

The evidence bearing upon the first point is briefly as follows; first, the sex cells always arise in, or attain by migration particular regions of the body in a particular organism, therefore, their physiological correlation with other parts cannot be purely nutritive in character, for if it were, there is no reason why they should not take the most various positions in the same species. Second, they undergo characteristic differentiations during the life of the individual, as do other organs and these differentiations begin at a certain stage of development of the organism, *i. e.*, at or near the end of the period of vegetative growth. This cannot be accounted for by quantitative differences in nutrition at different stages, because the growth of the primitive germ cells in earlier stages often requires very large amounts of nutritive material. If this development is predetermined, then physiological correlation between the germ cells and other parts must have existed at some earlier stage, or else we are forced to a hypothesis of pre-established harmony, which amounts to some form of vitalism.

Moreover, in organisms, which show both asexual and sexual

reproduction in the same individual, the asexual reproduction occurs earlier in the life cycle than the sexual, and in organisms which produce naturally both parthenogenetic and non-parthenogenetic eggs, the parthenogenetic eggs appear earlier than the non-parthenogenetic. In both these cases the earlier product is usually capable of a greater degree of regulation when it is isolated from the parent body, than the later; in other words, both the non-parthenogenetic egg and the sperm appear from their behavior to be more highly specified or differentiated than the asexual or parthenogenetic reproductive elements. There is then considerable evidence in support of the view that the history of the germ cells, like that of other organs, is in part the result of physiological correlation.

As regards the 'stimulus to development,' I have shown by experiment (Child, '11b) that in *Planaria* the process of reconstitution after physical isolation as well as extreme starvation followed by feeding, accomplish rejuvenation and that it is highly probable that various other factors bring about similar changes. And finally, I have considered the facts which indicate that the process of fertilization and the conditions inducing artificial parthenogenesis produce changes in the egg similar in character to the rejuvenation occurring in the other cases.

From this point of view, the stimulus to development of the egg is essentially a process or the beginning of a process of reconstitution and so is similar in its physiological effect to the factors initiating the various processes of asexual reproduction. Experimental reconstitution following section is then merely a special case of reproduction occurring under certain conditions, or we may say just as correctly that each form of reproduction in nature or experiment is a special case of reconstitution occurring under certain special conditions.

If this view be correct, then the fundamental problems of development and heredity are before us in every case where a physically or physiologically isolated part of an organism produces a new organism, just as truly as they are in sexual reproduction. In fact sexual reproduction constitutes the most complex case of all, but I am convinced that a recognition of its essential similarity to the

processes following experimental section and the physiological solution of parts is of the greatest significance for our conception and solution of the problems of inheritance and development.

#### CONCLUSION

It is sufficiently evident from what has been said that I consider the phenomena of regulation in organisms as constituting the essential characteristic of life as a continuing process. I agree with Jennings that the problem of regulation is the fundamental problem of life. All of our experimental investigations on living organisms are directly concerned with the problem of regulation in one way or another. In fact there are only two possible methods of investigating and analyzing the phenomena of life: one is concerned with regulation in the living organism, the other with the observation and analysis of the results of stopping the life-processes at this or that particular point, under these or those particular conditions. In the one case we observe and control the process in its action, in the other we seek to determine the effects of its past action. As we can watch the river at work and investigate the processes of equilibration resulting from alteration of its flow in one way or another, so we can investigate the living organism. And as we can stop the flow of the stream or divert it into other channels and determine something of what it has done along its course up to a certain time by examination of its channel, so from the dead organism, we can determine something of its past activity.

But the conclusions drawn from the examination of the channel of the 'dead' river are only fragmentary at best. Only by observing and controlling the river in action is it possible to acquire any adequate conception of what it really is. And so, I believe, with regard to the organism: the living organism will teach us more than the dead one though we must work with both. And when we work with the living organism we come at once face to face with the problem of equilibration, of regulation. And finally, I believe that the further our knowledge of the processes of equilibration in the organism advances, the greater will be the difficulty of finding an adequate foundation in biology for vitalistic or dualistic hypotheses.

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# PARAMAECTUM AURELIA AND PARAMAECIUM CAUDATUM

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ONE FIGURE

Leeuwenhoek in 1677 described<sup>1</sup> some "little animals longer than an oval" which he had discovered two years previously, and there is some reason to believe that this is the first published record of an organism belonging to the genus *Paramaecium*. The name *Paramaecium*, however, was first employed by Hill<sup>2</sup> to designate certain small organisms which were more or less oblong, in contrast to others which were round or decidedly vermiform, and either the present species *aurelia* or *caudatum* is probably the animal which he designated as 'Paramaecium species 3.'

Although Hill was the first to attempt to apply scientific names to microscopic animals, it remained for O. F. Müller to give a general classification of these forms, and to apply the Linnean nomenclature. He began this work on the infusoria as a section of a treatise entitled, *Vermium terrestrium et fluviatilium Historia*, which appeared in two volumes in 1773. Unfortunately he did not live to see the publication of his special work, *Animalcula Infusoria fluviatilia et marina*, 1786, which was edited by his friend, O. Fabricius. Müller described a *Paramaecium* and applied the specific name *aurelia* in the former of these works. In the latter work he described and figured<sup>3</sup> *Paramaecium aurelia*,

<sup>1</sup>Philosophical transactions, London, 11, 133. 1677.

<sup>2</sup>History of animals, 3, 1751.

<sup>3</sup>Plate 12, figs. 1-14.

together with several other forms, which at the present time are assigned to other genera. Müller's description is as follows:—

PARAMÆCIUM. Vermis inconspicuus, simplex, pellucidus, membranaceus, oblongus.

*Paramaecium aurelia*. Paramaecium compressum, versus antica plicatum, postice acutum.

Thus the organism described by Baker<sup>5</sup> as "Animalcules in pepper water, first sort," by Joblot<sup>6</sup> as Chaussou, by Ellis<sup>7</sup> as Volvox terebella, etc., received the name which, in spite of various vicissitudes, has come down to the present time.

The next great student of the lower organisms, C. G. Ehrenberg, in the first two of his treatises,<sup>8</sup> described several species of Paramaecium, and one of these is Paramaecium aurelia. In his third treatise<sup>9</sup> he described still another species which he named Paramaecium caudatum.<sup>10</sup> Five years later, in 1838, Ehrenberg brought out his monumental monograph, *Die Infusionsthierchen als vollkommene Organismen*, and in this work he described these two species as follows:<sup>11</sup>

*Paramaecium Aurelia*, Pantoffelthierchen.

P. corpore cylindrico, subclavato, antica parte paullo tenuiore, plica longitudinali obliqua in os multum recedens exeunte, utrinque obtuso.

*Paramaecium caudatum*, geschwänztes Pantoffelthierchen.

P. corpore fusiformi, antica parte obtusiore, postica magis attenuata.

Thus Ehrenberg described, on the basis of shape and size, the two common forms of colorless paramaecia which appear in

<sup>4</sup>Page 86.

<sup>5</sup>The microscope made easy, London, 1742. 3rd ed., 1744, p. 72, Pl. 7, fig. 1.

<sup>6</sup>Observat. fait. avec le microscope, Paris, 1754.

<sup>7</sup>Observations on a particular manner of increase in the Animalcula of vegetable infusions, etc. Phil. Trans., London, 1769.

<sup>8</sup>Abhandl. der Akademie d. Wissensch. zu Berlin, 1830, 1831.

<sup>9</sup>Ibid, 1833.

<sup>10</sup>Ehrenberg notes that Herrmann (Naturforscher, 1784) applied the name caudatum to a form which was probably a species of Amphileptus; also Schrank (Fauna boica, 1803) used the same name.

<sup>11</sup>Pp. 350-352. Pl. 39, figs. 6, 7.

modern systematic works as *P. aurelia* O. F. M. and *P. caudatum* Ehrbg.

Dujardin, in 1841, in his treatise on the Infusoria<sup>12</sup>, recognized but two species of Paramaecium as follows:

PARAMÉCIE AURÉLIE.—*Paramaecium aurelia*.

Corps ovale oblong, arrondi ou obtus aux deux extrémités, plus large en arrière.—Long de 0, 18 à 0, 25.

PARAMÉCIE A QUEUE.—*Paramaecium caudatum*.

Corps fusiforme, obtus ou arrondi en avant, aminci en arrière.—Long de 0, 22.

His figures of the two species show clearly the characteristic form which he considered diagnostic.

Various investigators, including Stein, and Claparède and Lachmann, questioned the justification of considering these two forms as distinct species, basing their opinions, as had Ehrenberg and Dujardin, solely on external characters, and they united these two forms under one species, and applied Müller's original name, *P. aurelia*. This union of aurelia and caudatum into one species was accepted by all the subsequent students of Paramaecium, e.g., Balbiani, Bütschli, Engelmann, Gruber and Kölliker and consequently all the early literature on the conjugation of this infusorian, refers to the organism as *P. aurelia*, although it had but a single micronucleus.

Maupas, in 1883, in his studies on the ciliates,<sup>13</sup> wrote:—

Tous les auteurs jusqu'ici ont décrit *Paramaecium aurelia* comme ne possédant jamais qu'un nucléole d'assez grande taille et mesurant de 0mm,005 à 0mm,008. C'est en effet la forme que l'on rencontre la plus fréquemment. Mais j'ai observé aussi de nombreux individus pourvus de deux nucléoles plus petits et de structure différente de la précédente. Ils étaient de forme sphérique et composés d'un corpuscule central opaque vivement coloré par les teintures et ne mesurant que 0mm,003; enveloppé d'une couche corticale mesurant en diamètre 0mm,005, claire et ne se colorant pas.

<sup>12</sup>Histoire naturelle des Zoophytes. Infusoires, etc. Paris, 1841. Pp. 481-483, Pl. 8, figs. 5, 6, 7.

<sup>13</sup>Contributions à l'étude morphologique et anatomique des Infusoires cilies, Arch. de zool. exp. et gen., (2), I, 1883, p. 660.

Thus Maupas tacitly accepted the current view that there was one large species of *Paramaecium*, but observed, for the first time, that certain paramaecia have a different nuclear apparatus from that previously described. This author, however, in 1888, stated that in his earlier work he, as all his immediate predecessors, had confused two species, and he wrote<sup>14</sup> as follows:

Ces deux formes de micronucléus constituent le caractère distinctif le plus important entre les deux espèces de Paramécies. La première forme appartient toujours et uniquement au *P. caudatum*, la seconde, également toujours et uniquement, au *P. aurelia*.

Pour Ehrenberg et Dujardin, *P. caudatum* se distingue par un corps allongé, fusiforme, obtus en avant, aminci en arrière: *P. aurelia* par un corps plus large, presque ovale, obtus aux deux extrémités. Ces différences de contour général, tout en étant réelles, ne sont pas absolument rigoureuses; car, si on ne trouve jamais de Paramécie à un seul micronucléus affectant la forme de trapèze obtuse, il n'est pas très rare d'en rencontrer à deux micronucléus, ayant pris la forme allongée à queue. Dans ce dernier cas, il est impossible de savoir à quelle espèce on a affaire, sans une préparation permettant de voir les micronucléus. Ce caractère distinctif, basé sur le contour général, n'a donc qu'une valeur relative. Il est cependant bon d'en tenir compte; car lorsqu'on s'est exercé à bien distinguer les deux espèces, il suffit presque toujours et trompe rarement.

Le *P. caudatum* paraît avoir une taille un peu plus grande que celle du *P. aurelia*. Ainsi, j'ai mesuré des premiers depuis 120 jusqu'à 325  $\mu$ , tandis que les seconds ont varié seulement entre 70 et 290  $\mu$ . En outre, *P. caudatum* se conjugue avec une taille variant entre 125 à 220  $\mu$ , et *P. aurelia* entre 75 à 145  $\mu$ . Pendant la conjugaison, le déroulement rubanaire, préparant la fragmentation du nucléus, s'effectue chez le *P. aurelia*, dès le stade D, tandis que chez le *P. caudatum* il ne commence que vers le milieu du stade G. Chez cette dernière espèce, le nucléus mixte de copulation donne naissance finalement à huit corpuscules, chez *P. aurelia* il n'en produit que quatre; il en résulte que chez celle-ci l'état normal se trouve rétabli dès la première bipartition qui suit la conjugaison, et chez *P. caudatum* seulement après la seconde.

Toutes ces différences anatomiques et physiologiques me paraissent plus que suffisantes pour justifier la distinction des deux espèces. Il

<sup>14</sup>Sur la multiplication des Infusoires cilies, Arch. de zool. exp. et gen., (2), 4, 1888, pp. 231-235.

est fort possible que Claparède et Lachmann aient eu raison, en considérant la forme *caudatum* comme plus typique que la forme *aurelia*. Si, en effet, on examine avec soin les dessins de O.—F. Müller, on penche à croire que le vieux micrographe a vu et figuré la première seulement. En se conformant strictement au principe de la loi de priorité, ce serait donc le nom *aurelia*, donné par Müller, qui devrait être conservé à la forme fuselée. Mais, d'un autre côté, Ehrenberg et Dujardin ont distingué ce type et l'ont dénommé *caudatum*. Si nous lui conservons la vieille dénomination *aurelia*, il devient impossible de transmettre le qualificatif *caudatum* à la forme qui, le plus souvent, est obtuse à ses deux extrémités. Il faudrait alors créer un nouveau nom. Je crois plus simple de conserver les dénominations d'Ehrenberg.

Since 1889, when Maupas<sup>15</sup> and Hertwig<sup>16</sup>, in studies on conjugation added further evidence for the distinction of the two forms, they have been generally accepted as 'good' species. Calkins, however, again raised the question in 1906: "I personally believe that the slight differences that distinguish the two types of *Paramecium* are not of specific value, and hold that *P. caudatum* should be regarded as a mere variant of *P. aurelia*."<sup>17</sup> He based this view chiefly on the following observations. One of a pair of ex-conjugants of *P. caudatum*, which he was studying by his well-known accurate culture methods, reorganized as *P. caudatum* and the other as *P. aurelia*, i.e., the latter had two small micronuclei, instead of one, and remained in this condition for about forty-five generations in pedigree culture, and then reverted to the *caudatum* type with one large micronucleus. While the *aurelia* phase existed, the rate of division was comparatively slow, and when the *caudatum* phase was reassumed the rate of division immediately increased considerably. Calkins also considered the relative size of the two forms, and the conjugation phenomena as described by Maupas and Hertwig, and concluded that these are not of such a character as to warrant their being considered diagnostic.

<sup>15</sup>Le rajeunissement karyogamique chez les ciliés, Arch. de zool. exp. et gen., (2), 7, 1889.

<sup>16</sup>Ueber die Konjugation der Infusorien, Abh. kgl. bayr. Akad. d. Wiss. München, 2, Cl. 17, 1889.

<sup>17</sup>*Paramecium aurelia* and *Paramecium caudatum*. Studies by the pupils of W. T. Sedgwick, 1906.

Jennings, in his studies on heredity in *Paramecium*,<sup>18</sup> showed that he could readily isolate a considerable number of pure lines from a wild culture, and that these pure lines breed true, i.e., there exist inherent hereditary differences in size, persisting when all other conditions remain the same. These different lines fall usually into two main groups, one group having a mean length greater than  $170\mu$ , and the other having a mean length less than  $140\mu$ . But he was able finally to isolate a line intermediate in size, and thus to bridge over the gap. As Jennings points out, even if it were not possible to isolate a strain of intermediate size between the two large groups, this would not give a basis for distinguishing two species. However, he states: "I may be permitted to add to the precise data thus far given a personal impression or surmise. Though, as I have shown, intermediate lines occur, I believe that it will be found that most *Paramecia* can be placed in one of the two groups that we have called 'caudatum' and 'aurelia'. In other words, if my impression is correct, most lines will have a mean length either below 145 microns or above 170 microns; rarely will lines be found whose mean falls between these values. Such at least has been my experience in a large amount of work. Furthermore, I am inclined to believe that those belonging to the smaller group (mean length below 145 microns) will be found to have as a rule two micronuclei; those belonging to the large group but one micronucleus. This matter is worthy of special examination."

Jennings and Hargitt in 1909 made this examination and in a preliminary communication<sup>19</sup> stated that "two sets of races could be distinguished, one set having two micronuclei, the other but one. The races with two micronuclei were all smaller than those with one. The larger races together thus correspond with what had before been described as *P. caudatum*, the smaller races with *P. aurelia*. The two differ also in the size, position and

<sup>18</sup>Heredity, variation and evolution in Protozoa. II. Heredity and variation of size and form in *Paramecium*, with studies of growth, environmental action and selection, Proc. Amer. Philosophical Society, 47, no. 190, 1908.

<sup>19</sup>Characteristics of the diverse races of *Paramecium*, Proc. Amer. Soc. Zoologists, 1909 meeting, in Science, March 25, 1910.

staining relations of the micronuclei, in ways that correspond to the descriptions of Hertwig and Maupas. But *in rare cases* specimens of the caudatum races have two micronuclei, those of aurelia races but one, thus confirming the observation of Calkins on this point."

In accordance with the conclusions of Calkins, I have used the specific name aurelia to include both the aurelia and caudatum forms; but my extended study of Paramaecia cultures has demonstrated that these two forms are remarkably constant, and I am inclined to the view that they are distinct species, in the sense in which this term is generally used in biological work. The data on which I base this conclusion are chiefly as follows: the pedigree culture of *P. aurelia* which I have had under daily observation for (so far) more than three and one half years, during which time more than 2100 generations have been attained, has bred practically true to the aurelia type as described by Maupas in the passage quoted. The pedigree culture of *P. caudatum* which I have carried for nearly seven months, and which has attained more than 300 generations up to the present time, has bred practically true to the caudatum type as described by that author.

The pedigree culture of *P. aurelia* was started on May 1, 1907, with a 'wild' individual which was found in a laboratory aquarium, and was carried on at Williams College during May and June, 1907; at the Woods Hole Marine Biological Laboratory during parts of the summers of 1907 through 1910; and at Yale University during the academic years from 1907 to the present time, November 30, 1910. The pedigree culture of *P. caudatum* was started on May 14, 1910, with a 'wild' individual collected from a pond at New Haven, Conn., and was carried on at Yale University except for a period of a few weeks in the summer when it was taken to the Woods Hole Laboratory.

The original specimen of each culture was placed in about five drops of culture fluid on a glass slide having a central ground concavity, and when the animal had divided twice, producing four individuals, each of these was isolated on a separate slide to form the four lines of the respective cultures. *The pedigree cultures have been maintained by the isolation of a specimen from*

*each of these lines practically every day up to the present time, thus precluding the possibility of conjugation taking place between sister cells. The number of divisions of each line has been recorded daily at the time of isolation and the average rate of these four lines has been again averaged for ten-day periods (cf. fig. 1). The culture medium has consisted of materials collected practically at random from laboratory aquaria, hay infusions, ponds, etc. The infusions were thoroughly boiled to prevent the contamination of the pure lines of the pedigree cultures by 'wild' individuals. Permanent preparations have been preserved from time to time for the study of the cytological changes during the life history.*

In the light of this experience with cultures I shall consider each of the characters emphasized by Maupas.

*Shape.* The general shape of the aurelia and caudatum forms is, in nearly all specimens, quite distinctive; aurelia is slightly more broad at the posterior than at the anterior end, while caudatum, as the name implies, is quite pointed at the posterior end as compared with the anterior end. The posterior end, in the specimens in my pure culture, is markedly pointed, and being free from endoplasmic inclusions, appears transparent and clearly delineated even under a lens with a magnification of ten diameters. I have been accustomed to allow stock material from my pedigree aurelia culture to multiply in large flasks of hay infusion, for various experiments on conjugation, etc. Frequently I have used this material for my elementary class in biology and I have found that even the novice has called attention to the fact that the shape of the ends was reversed as compared with the figure of caudatum in the text-book. McClendon, however, stated that in his study of aurelia and caudatum he found "no characters of outward form" which were diagnostic.

Changes in the vitality of my pedigree lines never have been very marked, and consequently I have not had organisms, in the direct lines of my pedigree cultures, representing physiological extremes to compare. Numerous experiments, however, have been made with 'stock' material left over after the daily isolations of the pure lines, which have clearly shown that, for

example, even when the aurelia and caudatum cultures are subjected to unfavorable environmental conditions, as, for example, scarcity of food, the very great majority of individuals retain the shape which is characteristic of the race.

*Size.* As has frequently been pointed out, size alone is an entirely inadequate character on which to base species. It is significant, however, I believe, that during the long life of my pure strains, I have never observed the relative size of the individuals of the aurelia and caudatum forms, when bred under identical conditions, to change greatly during any single period. Experiments have shown that even when the two forms have been bred under diverse conditions, for example, aurelia in a medium rich in food and caudatum in a medium with a very small amount of bacterial growth, the size of the caudatum form always has remained sufficiently great to render it distinguishable from the aurelia form. On the basis of size alone, then, it has been possible, with great accuracy, to separate the two forms when mingled together. It is probable, of course, that I began my pedigree cultures with very typical specimens of the aurelia<sup>20</sup> and caudatum groups as described by Jennings. If such be the case, then my cultures add considerable evidence in favor of the different strains which Jennings has isolated. It appears to me, however, that what that author has done for *Paramaecium*, can probably be done for many closely related species of infusoria, and the very fact that he did find it difficult to secure an intermediate race between the aurelia and the caudatum groups is a strong point in favor of the distinctness of the forms.

*Vitality.* It has been customary to regard the rate of reproduction of infusoria in culture as a just criterion of vitality. Maupas wrote:<sup>21</sup> "Cette faculté de reproduction (aurelia) ressemble beaucoup à celle de la précédente espèce (caudatum)." My cultures completely corroborate this statement, for during the six and one half months of the life of the caudatum culture, 324 generations have been attained, while during the same period,

<sup>20</sup>For further details of the culture see: L. L. Woodruff, Two thousand generations of *Paramaecium*; *Archiv für Protistenkunde*, 21, 3, 1911.

<sup>21</sup>Sur la multiplication des Infusoires ciliés, loc. cit., p. 234.

under identical conditions, the aurelia culture has advanced from the 1785th generation to the 2117th generation, or 332 generations. This gives a difference of only eight generations in the rate of reproduction of the two forms during seven months (cf. fig. 1). These cultures obviously do not support the statement, frequently made, that aurelia is a weaker form than caudatum.

Maupas remarked that *P. aurelia* was one of the most common infusoria, and Jennings found that a typical wild culture could

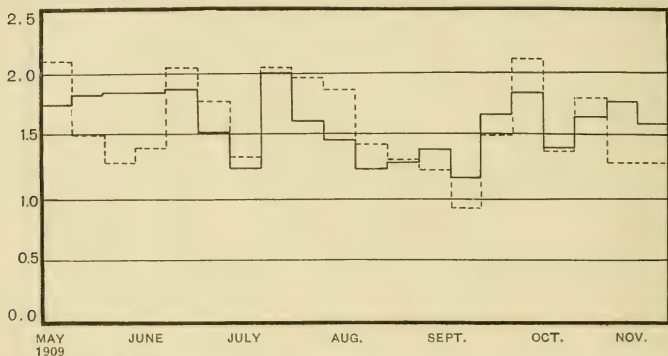


Fig. 1 Diagram showing the comparative rate of division of the pedigree cultures of *Paramaecium aurelia* and *Paramaecium caudatum*, when bred under identical conditions, from May 14, 1910, to November 30, 1910. During this period *P. aurelia* (designated by continuous line) advanced from 1785 to 2117 generations, while *P. caudatum* (designated by broken line) advanced from 1 to 324 generations. The rate of division is averaged for ten-day periods. The ordinates represent the average daily rate of division of the four lines of the cultures.

be resolved into caudatum and aurelia groups. It has been my experience that it is as easy to procure one form as the other in the wild state. Certainly my aurelia culture, which theoretically would provide individuals to the number represented by 2 to the 2117th power, gives more evidence of vitality and reproductive power than has been demonstrated for any other animal.

*Conjugation.* I have no data in regard to the conjugation of either of these forms, for, so far, in all experiments with stock

material left over after the daily isolations from my pure lines, I have failed to observe a single syzygy, either between aurelia lines or caudatum lines, or between aurelia and caudatum lines. Jennings's<sup>22</sup> experiments on conjugation in *Paramecium* bring out data which add further evidence that in certain strains at least a predisposition to conjugation does not exist. Maupas wrote: "C'est bien certainement une des espèces (aurelia) qui se recontrent les plus fréquemment à l'état conjugué."

Maupas, as we have seen, pointed out a difference in the nuclear phenomena during conjugation which he held to be of diagnostic value, and Hertwig apparently showed that aurelia has two micronuclei at the reorganization after conjugation. Calkins, on the other hand, has shown that *P. caudatum*, in one case, reorganized with two micronuclei and later reverted to the uninucleate type. Such a case can readily be considered a 'sport' which has arisen possibly by the persistence of the stage with two micronuclei immediately following the separation of the conjugants, or by the precocious division of a single micronucleus previous to the first regular vegetative division after conjugation. Although, as Calkins stated also, forty-five generations is a long time for an abnormality, if it be such, to persist; nevertheless, I believe it is very significant that, whereas during the presence of two micronuclei the division rate averaged only 0.8 of a division per day, after the loss of one of the micronuclei the division rate increased to the remarkable rate of 2.2 divisions per day, on the average for a period of four months. It is also of interest that the other exconjugant which reorganized 'normally' as caudatum failed to live.

So far as I am aware, the following statement<sup>23</sup> by Simpson is the only record of a possible case of conjugation between aurelia and caudatum: "Out of twenty-one attempts I had but two partial successes. Conjugation took place on two slides: the period was normal. After separation each of the ex-conjugates divided once: on the third day they died off. In anticipation of something

<sup>22</sup>What induces conjugation in *Paramecium*? Jour. Exp. Zool. 9, 2, 1910.

<sup>23</sup>Observations on binary fission in the life-history of Ciliata, Proc. Royal Soc. Edinburgh, 1901, pp. 407-408.

of this sort from analogy in higher forms, I intended to let the two pairs run their natural course, foregoing the desire to examine their nuclear condition. In view, therefore, of the incompleteness of the experiment, it is perhaps unwarrantable to draw any results regarding hybridization and infertility, or even the 'fixity of species' so far down in the animal scale." Simpson gives no data to prove that these were actually syzygies between the two forms, but if they were, it is obvious that they were not fertile. Jennings and Hargitt stated that they had been unable to induce the two forms to conjugate.

In view of the fact that, for example, Maupas studied conjugation of both *P. aurelia* and *P. caudatum*, and Hertwig studied conjugation of *P. aurelia*, and also that Jennings observed conjugation in both his *aurelia* races and in his *caudatum* races, it is clear that *aurelia* forms conjugate and *caudatum* forms conjugate, but there is no positive evidence that conjugation takes place between individuals of *aurelia* and *caudatum*.

*Macronucleus.* The normal macronucleus of *aurelia* was described by Hertwig and Maupas and that of *caudatum* agrees very closely. It is an ellipsoidal body with a smooth contour, except for a slight depression, in which the micronucleus is usually located. But the form of the macronucleus of both *aurelia* and *caudatum* frequently departs very greatly from the 'normal' condition. It is not unusual to find paramaecia of my *aurelia* cultures with the macronucleus resolved into several parts. These parts apparently may be gathered together into a typical nucleus for division, or the cytoplasm and micronuclei may divide, the macronuclear fragments which are in the posterior part forming the macronucleus of one daughter cell and those in the anterior part forming the macronucleus of the other daughter cell. I shall reserve the full discussion of these interesting changes for a special paper. It is important to emphasize the fact that these are not pathological conditions, since the general vitality, as indicated by the rate of division, is not appreciably affected.

Calkins,<sup>24</sup> however, found nuclear fragmentation in degenerating individuals of caudatum, Wallengren<sup>25</sup> and Kasanzeff<sup>26</sup> showed that various changes including fragmentation of the macronucleus occur when paramaecia are starved, and Popoff<sup>27</sup> described a large increase in size and fragmentation of the macronucleus in degenerating caudatum which paralleled the conditions observed in specimens ripe for conjugation. He also obtained similar changes by subjecting the animals to various reagents.<sup>28</sup> Mitrophanow<sup>29</sup> emphasized the fact that the structure of the macronucleus varied considerably under the influence of diverse conditions, and he described fragmentation and figured spherical pieces which very closely resembled micronuclei.

It is evident, then, from my cultures that the macronucleus of both aurelia and caudatum is subject to great morphological variation without appreciably affecting the rate of reproduction, i.e., it is entirely normal. It is also apparent from the work of the other authors cited that degeneration changes become manifest in the fragmentation of the macronucleus. Consequently the macronucleus presents no character which is of permanent diagnostic value.

*Micronucleus.* Maupas, as we have seen, regarded the micronucleus as the chief distinguishing character of aurelia and caudatum, and my cultures substantiate his view. Fixed and stained individuals show that the micronuclei of the aurelia culture for over two thousand generations have conformed in a remarkable degree to the aurelia type as described by the French investigator, and the micronuclei of the caudatum culture have conformed to his caudatum type.

<sup>24</sup>Studies on the life history of Protozoa. IV. Death of the A Series, Jour. Exp. Zool., 1, 3, 1904.

<sup>25</sup>Inanitionerscheinungen der Zelle, Zeit. f. allg. Physiologie, I, 1, 1901.

<sup>26</sup>Experimentelle Untersuchungen ueber Paramecium caudatum. Inaug.—Diss., Zürich, 1901.

<sup>27</sup>Depression der Protozoenzelle und der Geschlechtszellen der Metazoen, Archiv für Protistenkunde, R. Hertwig Festband, 1907.

<sup>28</sup>Experimentelle Zellstudien III. Ueber einige Ursachen der physiologischen Depression der Zelle. Archiv für Zellforschung, 4, 1909.

<sup>29</sup>L'appareil nucléaire des Paramécies, Arch. Zool. Exp. et Gen., (4), I, 1903.

It is not only the presence of two micronuclei, but their peculiar morphology, as emphasized by Maupas, which is characteristic of the aurelia type. I have found one individual of the aurelia culture with three micronuclei, and a few specimens in which I have been unable, in total mounts, to distinguish a single micronucleus or more than one micronucleus. But when only one micronucleus could be seen it has been of the aurelia type, and other individuals of the culture at the same period have had the two characteristic micronuclei. I have observed a variation in the number of micronuclei in various pedigree cultures of hypotrichs,<sup>30</sup> Popoff has found reduplication in *Stylonychia mytilus* and *Paramecium caudatum* during degeneration, and Kasanzeff has observed the same in starved *P. caudatum*. Thus, while my cultures of *Paramecium* and various hypotrichous species substantiate Wallengren's and Calkins' statement that the micronuclei are the most stable elements in the cell, and the last to be visibly affected by environmental changes, nevertheless it is apparent that they are subject to variations under certain unknown conditions. Temporary variation, therefore, cannot be considered as having weight in determining species. The essential fact is, however, that throughout the existence of my aurelia and caudatum cultures, the morphology of the micronuclei has conformed to Maupas' description for the respective species. It must be borne in mind also that *P. caudatum* has been the subject of more extended study by exact culture methods than any protozoon except *P. aurelia*, and in all these long pedigree cultures it has bred true to the caudatum type, at least with respect to the single micronucleus. Calkins, for example, in his important investigations on the life history of this form, carried three distinct cultures, by the aid of artificial stimuli during periods of physiological depression, through 379, 570, and 742 generations respectively. McClendon, also, studied mass cultures of *Paramecium* for considerable periods and stated that he never found individuals "with different numbers of micronuclei in the same culture."<sup>31</sup>

<sup>30</sup>An experimental study on the life history of hypotrichous Infusoria, Jour. Exp. Zool., 2, 4, 1905.

<sup>31</sup>Protozoan studies, Jour. Exp. Zool., 6, 2, 1909.

## SUMMARY

Briefly stated, I am convinced from my study of paramaecia that—

1. A very great majority of individuals of aurelia and caudatum can be distinguished on the basis of shape alone;
2. A very great majority of individuals of aurelia and caudatum can be distinguished on the basis of size alone;
3. The power of reproduction, or general vitality, of aurelia and caudatum is practically identical;
4. The macronucleus of aurelia and caudatum is subject to such great variation that it affords no diagnostic feature;
5. The micronuclear apparatus of aurelia and caudatum affords crucial diagnostic characters.

I have summarized the various characters of the two forms as they have shown themselves in my long pedigree cultures, and it is evident that they have conformed practically identically to the Maupasian types—such variations as have appeared not being so great as have been observed to occur in undisputed species, or as one would expect to find when the intimate relation of the unicellular organism to the environment is considered. Therefore, I believe, that since one of the crucial tests of a species is its ability to breed true to its type indefinitely, aurelia and caudatum have adequately met this test during more generations than any other animal under observation, and accordingly *Paramaecium aurelia* O.F.M. and *Paramaecium caudatum* Ehrbg. should be regarded as distinct species.<sup>32, 33</sup>

<sup>32</sup>In this paper I have followed the spelling of the name of the genus as given by its founder, except in direct quotations from other authors.

<sup>33</sup>I have the satisfaction to note that my conclusions are in accord with the final results published by Jennings and Hargitt in the last number of this journal, which was received when this paper was in press. Hargitt says, "There is cytological warrant for distinguishing caudatum races from aurelia races, and it seems probable that it will continue to be convenient to distinguish these as two species."



# MALE ORGANS FOR SPERM-TRANSFER IN THE CRAY-FISH, CAMBARUS AFFINIS: THEIR STRUCTURE AND USE

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THIRTY-ONE TEXT FIGURES AND FOUR PLATES

## INTRODUCTION

The present paper is a contribution to our knowledge of the means that lead to the fertilization of the egg. It is part of the history of the sperm outside the body of the animal.

Sexual reproduction in most complex animals involves the transfer of the sperm from one animal to another, before the eggs can be fertilized.

Among animals the various methods by which the sperm is transferred may be grouped under the three heads, diffuse, direct, indirect. By diffuse sperm transfer we mean the discharge of the sperm into the water, where it may meet the eggs outside of the female, as in certain coelenterates, echinoderms and annelids, or may be drawn into the body of the female, as in certain lamel-libranchs. By direct sperm transfer we mean the method found in the majority of complex animals, in which there is more or less direct application of the terminal parts of the passages leading the sperm to the exterior to the passages leading from the exterior direct to the eggs. In this group there is commonly a true copulation.

By indirect sperm transfer we mean those peculiar complex methods of getting the sperm from the testis to the eggs that are found in a few cases amongst the great groups of animals, as in

earthworms, spiders, some cephalopods and leeches. The essence of these cases of indirect sperm transfer lies in the fact that while the sperm is transferred by organs, and not by floating, yet these organs either do not put the sperm into the egg passages, or else if they do they are not organs directly concerned with the discharge of sperm, or both may be true. In indirect sperm transfer there is no true copulation, or intromission, but at most conjugation or clasping.

The three methods are not always sharply separable, and may be regarded as only convenient groupings of physiological processes that occur here and there among animals without reference to their systematic positions. The diffuse method is obviously the one open to the most hazard in the sperm and eggs meeting; the direct method by intromission the best assured method; the indirect method most peculiar and needing special explanation in each case.

In the crayfishes and lobsters most interesting cases of indirect sperm transfer occur, and it is the purpose of the present paper to describe the organs of the males that are used to transfer the sperm to the receiving organs that have been described in previous communications (1, 2, 3). In these animals the male transfers the sperm to the outside surface of the female where it remains till the eggs are laid, when fertilization takes place outside the body. In the American lobster and the sixty and more species of crayfishes of the genus *Cambarus* that are found in all but the most western parts of North America, the sperm on the shell of the female is stored in a special pocket, or receptacle, but in the other genera of crayfishes, all the world over, there is no such receptacle and the sperm is believed to be distributed over the shell of the female in separate spermatophores. While the sperm pocket has been described (1, 2, 3) the organs of the male that fill the pocket have had only such consideration as was necessary for the systematist, who found them to be of the greatest value in distinguishing species and in forming subgenera.

In the present paper the anatomy of the male organs is examined and their use as organs of sperm-transfer is explained.

## CAMBARUS AFFINIS

While the sexual habits of all the species of *Cambarus*, agree in the main, the species *affinis* has been more studied, and as in describing the female organs concerned in sperm transfer we first considered this species, we will also give chief attention to the male organs of sperm transfer in this species.

As elsewhere described (4, 5) conjugation is here a long series of activities of the male accomplishing the accurate adjustment of the essential transfer organs of the male to the receptacle of the female. The receptacle of the female is a single pouch in the shell, but the transfer organs of the male are three pairs of outgrowths. On each side of the body there is a papilla, or special termination of the sperm duct, and two limbs, those of the first and second segments of the abdomen, which we may call the stylets.

To introduce the sperm into the receptacle the papilla must be adjusted both to the first and to the second stylet and both sides of the body play a necessary part in the process of sperm transfer.

## THE PAPILLAE

One external instrument concerned in the process of sperm transfer is the modified end of the sperm duct that emerges from the base of the fifth leg, on each side of the animal. These organs are the papillae.

Since systematic work has been done largely upon preserved specimens it is not so generally known that the sperm duct ends in life, in a soft, turgid protuberance, which may be so collapsed after death as to leave only the rounded hole in the firmer shell as the apparent ending of the sperm duct. These papillae lie concealed by the stylets, at rest, but on raising the stylets the papillae are seen as conspicuous, clear, tubes about 3 mm. long and  $1\frac{1}{2}$  mm. wide, jutting out from the base of each fifth leg.

At the time of conjugation the papillae are also concealed from view since the necks of the first and the second stylets form a nicely adjusted frame about the papillae and this frame is fitted in between the bases of the fifth legs. Certain in and out move-

ments of these bases seem to adjust the papillae so that they fit accurately into the orifices of the first stylets (figs. 30, 31) and by that means the sperm discharged from each papilla is passed into the cavity of the stylet.

The papilla (*P.* fig. 1) is on the under side of the large first segment of the leg and projects downward and toward the median plane; but its tip turns away from the middle line of the body. The papilla is a cone with bent apex. It is translucent and distended with colorless blood. When directly injured, or upon lessening of the blood pressure from injury elsewhere, the papilla collapses, being but a thin uncalcified protrusion of the skin, kept turgid, or erected, by blood pressure. Within the papilla one can see a large central tube passing toward the tip and also chalky white masses suspended between the central tube and the thin outer walls.

On the shell at the base of the papilla there is, anteriorly, a single row of very long setae (fig. 1) that form a sort of protective screen over the anterior face of the papilla.

Sections show that the papilla is a continuation of the deferent duct, blood cavity and skin, so constructed that the bent, conical apex, with its soft walls can be adjusted to the hard opening of the stylet so as to fit hermetically, as a tense rubber bag might. Moreover the bent tip can be opened to discharge the sperm, when special muscles remove the obstructing valve that holds the tube closed.

A lengthwise section through this delicate papilla (fig. 2) shows that the central tube is a direct continuation of the deferent duct that leads the sperm from the testes to the tip of the papilla. Between this duct and the outer cuticle there is a large space full of blood, traversed by little connective tissue and in it are the white bodies just mentioned, now seen to be small tubular glands, opening into the central duct. The central duct presents two strikingly distinct parts; the one continued from within the leg has the thick muscular wall and peculiar secreting lining of the deferent ducts, the other is lined by the thin cuticle inflected at the orifice at the tip of the papilla, and lacks muscle. In place of muscle the wall has only epidermis, which extends irregularly



Fig. 1 Posterior face of the left fifth leg of a living male 95 mm. long to show the translucent papilla. (P.) 2. *ao.*

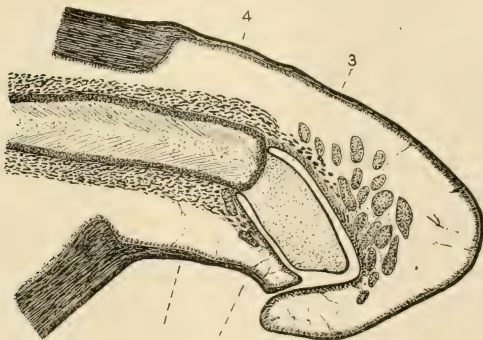


Fig. 2 Longitudinal section through the papilla and the base of the fifth leg, showing the orifice of the sperm duct, the valve, the muscles and the glands. 2. 90 mm. A.

into the blood space as the tubular glands alluded to above. The orifice at the tip is small and is not closed by any muscle, but apparently by blood pressure only. The part of the tube lined by cuticle has its lumen much reduced by a valve, or great longitudinal ridge, which extends out as far as the abrupt bend at the orifice. In a cross section (fig. 3) this ridge is well seen, as is also the fact that some muscle fibres run into it and that the glands are chiefly on the side opposite the ridge. The ridge appears to act like a valve to hold this part of the tube closed, while contractions of the muscle would tend to open the tube wide and let the sperm pass to the orifice, which would then be forced open by the internal pressure of the sperm squeezed by the muscles of the wall all along the length of the duct, or some extent of it at all events.

The upper part of the duct, as seen in the cross section fig. 4, has its thick muscles arranged chiefly in transverse fibres and is lined by an epithelium that evidently in large measure breaks down to furnish a great mass of secretion about the sperms. It is probably this secretion that envelops the sperm in the form of macaroni-like tubes, when they pass out in a slow stream.

#### THE STYLETS

The most complex of the organs concerned in sperm transfer are the modified limbs of the abdomen which we will call the stylets. In the male the sixth pair of abdominal appendages form the large side parts of the tail fan while the third to the fifth inclusive are the simple and apparently rather useless swimmerets. The first and second pairs are specially constructed to serve as transfer organs for the sperm.

These appendages of the first and second somites are much stouter and longer than the following swimmerets and have a very firm attachment to the abdominal sterna. The calcified ridge across the middle of the sterna is much more developed in the first and second somites, and where the appendages are fastened it rises up as a decided elevation which remains as a stump when the appendage is cut off. On the second somite these stumps are far apart, (some 10 mm. in a male of 100 mm.) while on the first

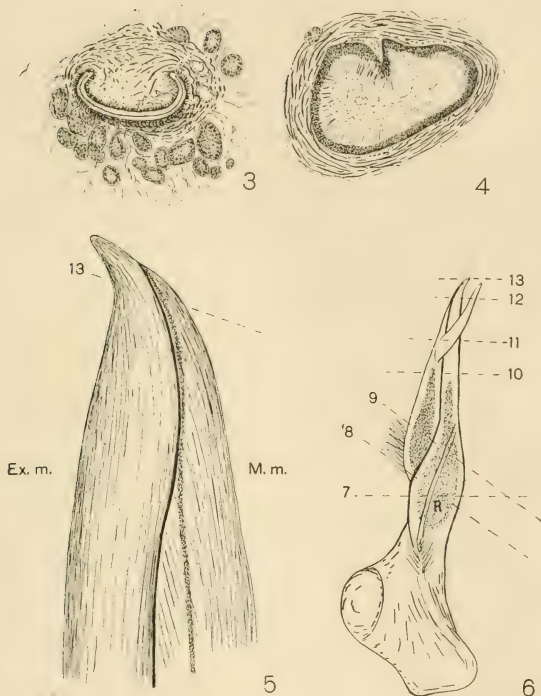


Fig. 3 Cross section of the sperm duct and valve along the line 3 of fig. 2, showing the duct closed by valve ridge. 2. A.

Fig. 4 Cross section of the sperm duct along the line of 4 of fig. 1, showing the muscular wall and the lining epithelium disintegrating in secretion. 2. A.

Fig. 5 Extreme tip of right first stylet, showing the groove bottom coming to the surface, posterior face. 2. A. *Ex. m.*—the external mass. *M. m.*—the internal mass. 13—the level of the section, fig. 13.

Fig. 6 Diagram of stylet as in plate I, fig. 1, to show location of glands in the interior, and the location of the sections, 7 to 13, shown in figs. 7 to 13.

somite they are in contact at the median line of the abdomen. The elliptical transversely elongated stumps of the first appendages are 5 mm. long and those of the second about 3 mm.

Commonly these appendages are carried forward horizontally under the thorax between the thoracic legs in a deep depression of the thoracic sterna. The first pair lie close side by side with their median faces in contact. The second pair lie over and largely conceal the first, since their form enables them to come to the middle of the body beneath the first pair in spite of the fact that their bases are attached to the sterna, so far from the middle line.

In a dead male one may move the appendages upon their attached bases as follows:

The first may be moved upon its base from the horizontal up toward the vertical only about  $45^{\circ}$ . The membrane on the anterior face of the joint at the base of the appendage is stretched to its limit when the appendage is pulled up a little beyond sixty degrees, so that this appendage is never vertical and cannot swing back and forth through a wide arc as do the ordinary swimmerets. The distance traversed by its tip is some two cm. The appendage may also be rotated a very little at its base and moved from side to side a little so that its tip travels some 5 or 6 mm.

The apex of the second may be drawn from the horizontal up a little beyond the vertical; but neither the basal protopodite nor the endopodite travels more than  $90^{\circ}$ . They are set together at a large angle, so that while the main length of the appendage is horizontal the basal part never is, and when the base goes back some 90 degrees the horizontal part is swung past the vertical line. The tip traverses some 3 cm. The base may be rotated a little and moved from side to side so that the apex travels 6 or 7 mm.

#### STRUCTURE AND ANATOMY OF THE FIRST STYLET

The first abdominal appendage of the male is a very stiff calcified mass of the general shape of an awl, some 17 mm. long, but having two tips. There is a groove along more than half its length and the base is articulated to the ventral shell of the animal so

that the appendage has very little mobility back and forth through some  $45^{\circ}$ . The normal position of the stylet is pointed forward under the thorax, where it lies horizontally in a deep groove, but in use it is dropped down and backward toward a vertical position. It has an anterior face, which is usually carried as the dorsal side, a posterior face which is usually the ventral aspect, and an outer and an inner or median face.

The general appearance of the stylet is seen in the photographs, figs. I, II, III, IV, which represent respectively the posterior, median (or rather median and posterior somewhat diagonally), the anterior and the outer faces of the same left stylet. Fig. I, the posterior face, is the view got by looking at the under side of the crayfish, after lifting up the second stylet, which lies over the first and largely conceals it.

The first pair of stylets do not spring from the sternal surface far apart as is the case with the common, unmodified swimmeret, but they arise very close together; in fact the median faces, (fig. II,) of the two come into contact so that these two appendages really form one mass. If looked at from the dorsal side, the two are seen to lie in contact at the base and all along the distal half, leaving between the constricted parts of the two a square opening that is occupied, in rest, by part of the second stylet.

In describing the stylet we will distinguish the base, the neck, and the scroll or spiral that contains the groove. The scroll ends in two tips, the more slender, side outgrowth, or spatula, and the real end bearing the groove, the canula

The base is some 6 mm. wide and long and only 2 thick, being flattened from before back. The posterior or ventral face of the base, fig. I, presents a wide groove bounded on the median side by a rounded knob and on the outer side by a long ridge which, as it passes on to the neck, bears a tuft of long, finely plumose setae, that are seen again in profile in figs. II, IV. In this deep groove the second stylet lies when not in use, so that the two appendages are firmly packed together under the thorax of the male.

The part of the base joined to the sternum of the animal is an oblique elliptical area, around the edge of which the hard shell gives place to the soft articular membrane that makes it possible

to cut the whole appendage away from the sternum. In this membrane there is an articular, whitish plate that is seen in figs. III and IV. The whole base is pyramidal and except the posterior all its faces (figs. II, III, IV) are convex and rounded.

The neck is the narrowest part, before the sudden enlargement of the spiral part; it is the smallest of the three regions; and is best seen in figs. II, III, IV. The neck passes gradually into the base and ends abruptly at the spiral. It is some 3 mm. long and 2 wide and thick. It has an angle along the ventral face that continues the ridge of the base up to the outer part of the spiral.

The spiral or scroll may be likened to a long triangular plate with its edges rolled in together so as to leave a groove between them, but it is a plate some 8 mm. long, with the edges greatly thickened, so that the resulting mass is apparently solid. The groove begins on the median side, fig. II, and passes in a sinuous course to the ventral side and along this diagonally to the very tip. The apparent bifid nature of the stylet is due to an outgrowth from the median part, quite separate from the real end of the organ, in which the groove is continued through its entire length. We have then to describe a sinuous groove and its two boundaries, which we will call the median mass and the external mass; and also the two tips. The external mass, seen from the ventral side on the right of fig. I, shows a proximal part about 2 mm. long and 1 mm. wide, bearing a marked ridge parallel to its sides and continued up from the neck. And then it suddenly turns at a large angle and becomes a rounded and gradually tapering terminal part, something less than  $\frac{1}{2}$  mm. wide at first, and 6 mm. long. This passes behind the slender protuberance of the median mass to end as a flattened, horny tip together with the like ending of the median mass. In other words both external and median masses unite as the horny tip that we will call the canula. The sudden change in direction of the mass is accompanied by a like change in the groove whose edge it forms; this change of the groove we will call the angle of the groove.

Seen from the outer face, fig. IV, the external mass is widely swollen proximally, some  $2\frac{1}{2}$  mm. deep, and gradually narrows into the distal part. The round canula is bent somewhat, ventrally.

On the dorsal face, fig. III, the external mass is confluent with the median mass, without boundary line. Thus the distinction between the two masses is useful chiefly on the anterior face where they form the two sides of the groove.

In fig. III, the long triangular region running from the notch that marks off the neck from the spiral region and ending distally in the rounded and pointed canula, is to be regarded as made up chiefly of the median mass, but the depressed part along the left edge is part of the external mass.

On the median face, fig. II, (which is unfortunately turned so that part of the posterior face shows) the external mass shows only its proximal end along the side of the diagonal groove, and into this groove the external mass here sends a narrow horny shelf, dimly seen as light in fig. II. The external mass has an angular projection, or lip, at the very beginning of the groove which will be described in connection with the orifice of the groove. At the tip, part of the external mass is seen making the lower part of the canula, to the right, that is, the curved strip of external mass seen is flat and on a lower level than the median mass.

On the ventral face, fig. I, the median mass looks like a long rounded white bone that begins suddenly without apparent connection with the neck and, after running nearly straight for some 6 mm., turns externally across the external mass as a flat, curved process that we will call the spatula. Beyond the spatula, which stands out freely as the second tip of the appendage, the median mass continues as the narrow median edge of the canula. From the external view, fig. IV, the visible part of the median mass, the spatula is back of the external mass.

In the dorsal view, fig. III, the main part of the spiral region is median mass, forming a long triangle, beginning at a deep notch near the neck and extending in the foreground as the visible part of the canula and back of that as the spatula. At the notch may be seen part of the lip on the external mass.

The median face best shows the median mass, but, fig. II, being not an exactly median view, does not do justice to it. In reality this face is markedly flat where it comes against the like face of of the other stylet of the pair. This flat face is a long ellipse, 2

mm. wide and 5 long, and is smooth except for a roughened area near its proximal end where there is a long tuft of finely plumose setae which bend abruptly downward, that is, posteriorly, as if an adjustment to the fact that they are pressed in between the two stylets. These setae are so long as to be visible from all points of view, cf. figs. I, II, III, IV.

The groove itself is seen only from the median and ventral views. It is some 7 mm. long and begins as the orifice on the median face where it meets the ventral, fig. II. The orifice is a conical opening bounded by that depression of the neck that makes the notch so conspicuous in fig. III, by the rounded origin of the median mass, fig. II, and by the overhanging lip of the external mass. It is of such shape that the tip of the spout, fig. 1, can fit into it. The groove leads from the orifice obliquely outward and distally between the external and median masses some 3 mm. and then turns to make a rounded angle, fig. I, toward the median line some 3 mm. more. In this part of its course it is soon concealed behind the median mass that is rising to form the base of the spatula, but it still exists there and emerging again runs the entire length of the spatula as a very narrow slit with horny edges. The groove is thus a long double curve, bending abruptly outward, then forward and slightly inward and finally outward again, as seen from the ventral side. But it also bends in the vertical plane, passing downward, then forward and upward and finally a little downward at the tip. While the walls of the groove seem to be merely hard rounded bone there projects into the groove from the side a narrow shelf of horn that springs from the external mass only. This will be seen in sections.

The spatula is a flat flagellum-like process some 2 mm. long,  $\frac{1}{2}$  wide and perhaps  $\frac{1}{8}$  to  $\frac{1}{10}$  thick. It is curved and pointed as seen in the figures. It springs from the median mass where this suddenly narrows to help form the canula, fig. II. In life the spatula is milky white and pliable, not bony, more like leather. At its base it passes suddenly into the bony walls of the median mass and there can be bent as if in a socket. After drying it looks more like a thin chitinous membrane over a dried contents. It is somewhat concave at the base on the dorsal face. With methyl

green the horny tip of canula and the shelf in the groove stand out clearly as distinct from the substance of the spatula.

The canula is some 3 mm. long and at base  $\frac{3}{4}$  mm. wide and thick. It is a long cone, flattened somewhat from before back, bent upward dorsally, and ending in a rather sudden point that bends outward from the median side. The canula is made up of both external and internal masses. Most of the length of the canula is clear, yellow, horny matter, but at the base this is continued as the white calcified material of the rest of the stylet. The bone of the external mass stops rather suddenly, while that of the median mass is continued in the midst of the horny cap as a central area, as seen from the median view. An enlarged view of the tip of the canula, fig. 5, shows that both external and internal masses make about the same amount of the canula, since the groove continues sinuously almost to the exact tip of the organ, but yet there is a greater prolongation of the external mass to form a short ungrooved apex. This sketch is from a canula of the opposite side of the body from that in fig. 1. The two canular tips flare away from one another.

The groove may be said to begin and to end on the median face and to be shoved away from it through most of its course by the ridge that we have called the median mass (fig. 1.).

#### INTERNAL ANATOMY

When the stylet is macerated some days the entire contents may be drawn out of the hard shell; such a cast of the shell has its general long conical form with a short conical tip that came out of the canula and a short flat plate that came out of the spatula. It is made of connective tissue and blood covered with epidermis with some red pigment cells and shows at the base some muscles and at the middle some glands.

The muscles, as made out by dissection of fresh and preserved crayfishes, are weak and run from the base of the stylet into the adjacent ridge of the sternum upon which the stylets articulate. There is a wide thick fan of muscle that passes from the bony articular plate of the anterior face of the stylet, fig. III. When

this is pulled the stylet is raised dorsally into its position of rest. Since it lowers the organ into the groove on the thorax it may be called the depressor, though it really swings the appendage forward.

This depressor muscle is lodged in the protruding ridge of the sternum from which the stylets spring, and its fibres are made fast to the posterior wall of this ridge. There is also a smaller muscle attached to the base of the stylet at its external edge which would seem to antagonize the other and to tend to swing the stylet backward, that is, to raise it up from its horizontal position of rest into the erect position of use; it may be called the erector muscle.

The internal anatomy of the stylet as well as the character and mode of use of the groove, were made clear from sections.

The diagram fig. 6 shows the ventral view of a left stylet as if transparent, the extent of the glandular area being shaded; the glands occur in both external and internal masses, but not in the base of the stylet, and they extend from the neck to near the origin of the spatula, filling most of the cavity of the region in which they occur.

The sections, (figs. 7 to 13, inclusive), were taken across the stylet along the planes indicated by the like numerals in fig. 6.

The transverse section, (fig. 7) shows in black the exceedingly thick shell with the depression on one side that forms part of the orifice of the groove, overhung by the solid lip. Through the thickness of the shell that forms this part of the orifice are seen many fine tubes, passing from the internal glands to discharge on the surface. The interior of the stylet is a delicate mass of connective tissue, chiefly blood sinuses, crossed by few strands of tissue, and bounded by the thin epidermis against the shell. Scattered all through this are the tubular glands that bend and are cut at various angles. These glands ultimately discharge by the numerous fine ducts that penetrate the shell. In this section the sharp angle above is the ridge (*R*) seen in figs. 6 and I passing along the external mass. The angle to the right is the line between the ventral and external faces of the external mass.

Sections 8 and 9 show the orifice passing into the groove; they are cut obliquely transverse and, in addition to the section of the first stylet, show also the section of the second stylet as it lies locked in the first. Disregarding for the present all but the lower part of the sections we see that the stylet has widened out from the constricted neck into a wide flattened mass sub-divisible with reference to the groove into the external and median masses. In

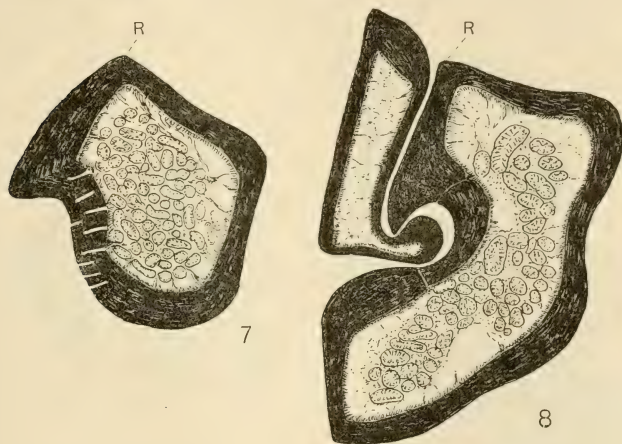


Fig. 7 Section across the stylet, in the region of the neck, just below the orifice, on the level 7 of fig. 6. *R*—the sharp ridge on the external mass, fig. 6 and 1. 2. 90 mm. *A*.

Fig. 8 Cross section of the same at the level 8, showing the groove above the orifice filled by the head of the accessory stylet, which is the separate mass lying to the left and above. 2. 90 mm. *A*.

fig. 8 the orifice is so overhung by the lip as to be in section a C-shaped bay, embracing the head and neck of part of the second stylet. Here again the shell is remarkably thick, but is penetrated by the ducts of the glands discharging on the surface that lines the orifice. In fig. 8 the lower straight side to the left is the flat face that is normally applied against its fellow on the outer side of the body. Above is the angle (*R*) that represents the ridge of

the external mass, just as in fig. 7. In the interior some of the glands are very large. The section distal to this, (fig. 9) shows the bottom of the groove receded from the surface and constricted from the rest by the continuation of the lip so that it forms a rather elliptical hole with only a very narrow slit opening into the deep groove that is seen from the surface. This surface groove is bounded on the left by the greatly thickened shell substance of the median mass and on the right by the thick shell of the external

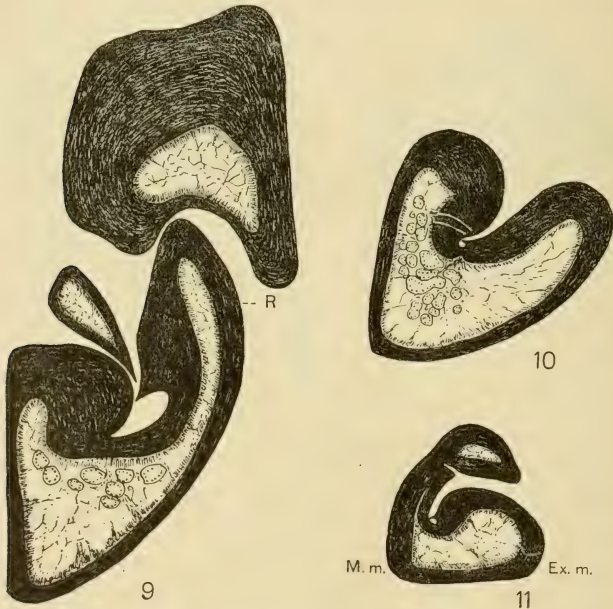


Fig. 9 Cross section of the same at the level 9, showing also the grasping second stylet, above, and its wedge, to the left, where it is entering into the groove of the spiral. 2. 90 mm. A.

Fig. 10 Cross section of the same at the level 10, showing the bottom of the groove cut off by the shelf from the external mass. 2. 90 mm. A.

Fig. 11 Cross section of the same, at the level 11, showing, above, the base of the spatula. 2. 90 mm. A.

mass. The cavity within the shell of the external mass is reduced to a narrow space and the glands have become few.

Further along the stylet, (fig. 10) the groove has passed from opening to the left (fig. 8), through the position shown in fig. 9, to open more toward the right. The groove is a deep and narrow one. Into it still open some few gland ducts from the remaining glands of the median mass. As before the side walls of the groove are made of very thick shell. The most unexpected fact is that the bottom of the groove is shut off as a very minute hole overhung by the continuation of the lip, which is now a horny shelf passing all along the groove, near its bottom, and so nearly meeting the opposite side as to practically shut off the bottom of the groove as a special tube. This figure shows the form of the stylet at the level, 10, of fig. 6. The flat side to the left is the flat face of the median mass, while the rounded edges of the groove are the two narrow parts of the external and median masses seen from the ventral side in figs. 1 and 6, just proximal to the base of the spatula.

A section through the base of the spatula, (fig. 11) shows the groove above overhung by the rising spatula that conceals it from surface view, (figs. 1 and 6) but still allows access to the groove from the right, in under the spatula base. The external mass (*Ex. m.*) is now the greater, but it contains no glands, while the median mass is reduced to a nearly solid shell prolonged as the slightly hollow spatula. The tube at the bottom of the groove is still there, overhung by the little chitinous shelf.

Near the apex of the organ, (fig. 12) the groove is again open above, as we have passed beyond the base of the spatula, only the tip of which is cut, lying well over to the right. This figure being magnified twice the diameter of the preceding figures, shows plainly the shelf that cuts off the bottom of the groove. The median mass is a narrow and nearly solid shell that forms the left wall of the straight, deep groove. The external mass is the main part of the section and contains much very watery connective tissue, covered with epidermis. In this section, the calcified part of the shell is represented in black, as in the other sections, while the chitinous or horny parts are dotted. From the surface this region of the canula looks to be only chitin. Farther on the

calcified part of the shell fades away and only pure chitinous matter is left, so that a section at the very tip of the canula, (fig. 13) is only chitin. This view is enlarged four times as much as the preceding one and shows the disappearance of the superficial part of the groove though the bottom, which is now close to the surface, is still overhung by the shelf from the external mass. That is the tube at the bottom of the groove can now discharge by a slit to the surface at the tip of the canula; see fig. 5, where the surface slit of the groove is represented by the black line and the bottom of the groove, or the tube, is represented by the dotted line, which comes finally to the surface at the tip as seen in the section across the level 13. As fig. 5 is of a right stylet and the section 13 from a left stylet it shows the parts reversed; the main bulk of the section is really of the external mass, as in fig. 12.

The specialization of the bottom of the groove had not been expected till sections revealed it and suggested some special use. Sections of stylets taken when being used in conjugation soon showed that the tube at the bottom of the groove is the channel for the transfer of sperm. Along this minute tube all the sperm passes from the papilla to the sperm pocket of the female. A section across the stylet where the median surface bears a tuft of setae, between the levels of 8 and 9 of fig. 6, when sufficiently enlarged, shows that the sperm is contained inside the tube of the groove, as in fig. 14. This shows only the part of the shell about the tube, with the sharp edge of the shelf above, jutting out to almost meet the wall of the median mass (see fig. 9). The cavity of the tube is full of a secretion containing at its centre a pear-shaped mass of the peculiar sperm of the crayfishes. As was shown (6) these sperm do not assume the star shape they have in books as long as they are in the male and not even when in the sperm receptacle of the female when normally protected from the water, and in this section, where they are seen in transit, they are still spherical, clear bodies with the peculiar bowl-shaped central part that, as represented in the sketch, might be thought a central nucleus. All along the groove above the orifice there is thus a strand of sperm surrounded by a paste-like white mass that fits tightly into the tube.

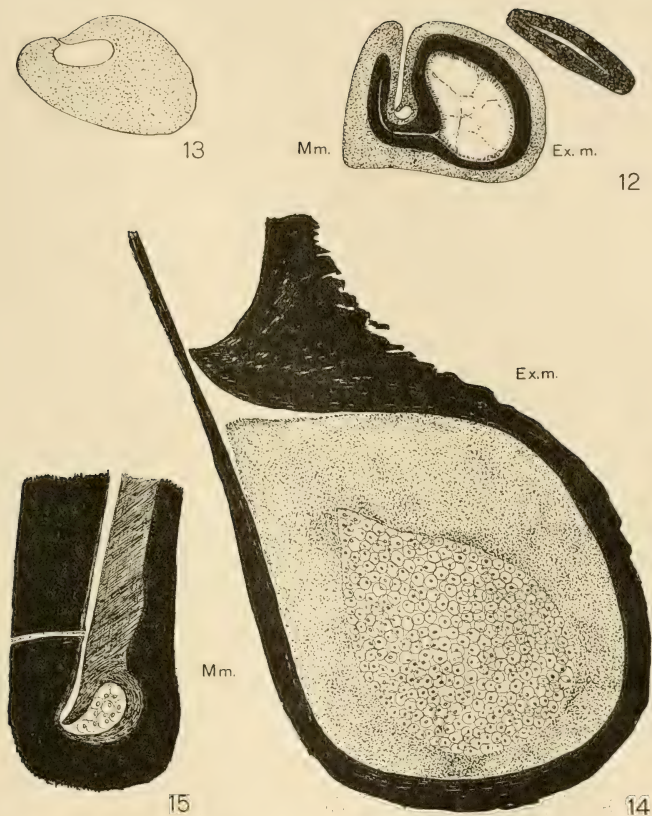


Fig. 12 Cross section of the same near tip, at level 12, showing the spatula cut off to the right. 2. A.

Fig. 13 Cross section of the very tip of the stylet, at the level 13, of figs. 5 and 6, showing the groove coming to the surface of the horny canula. 2. D.

Fig. 14 Enlarged view of section across the tubule, between the levels 8 and 9 of fig. 6 showing the sperm cells enveloped in a secretion and shut in by the shelf above. 2. D.

Fig. 15 Enlarged view of section of tubule and bottom of groove, about the level 10 of fig. 6 showing the fewer sperm and little secretion in the tubule, surrounded by a thick horny layer. The calcified skeleton is represented as black. 2. D.

That this mass is run in under pressure seems indicated by the way it tends to flow out at the narrow slit leading up from the tube into the groove and by the form of the sperm mass that tends likewise to copy the shape of the cavity that is filled, being pointed toward the slit (fig. 14). In successive sections this sperm mass is found all along the length of the groove, always in the bottom of the tube only, while the enveloping secretion for the most part disappears. Thus in the fig. 15 from the level 10, where there are still some secretion tubes coming through the heavy shell of the median mass, (fig. 10) there are a dozen or so sperm enclosed in the minute tube together with very little secretion and the sperm seem to come into contact with the shell. At this level, however, the thick and well-calcified shell (fig. 10) is covered by a thick layer of horny substance that makes the shelf and continues on up the face of the external mass bounding the groove, (fig. 15). The discharge of the milk-white sperm from the tip of the canula, (figs. 5, 6 and 13) was seen in some males separated from females in conjugation.

The anatomy of the stylet thus shows it to be a more refined and specialized tool for sperm transfer than had been expected. It is essentially a very fine tube receiving sperm at its larger base and discharging it at its attenuated tip; but it has walls that give it great strength and rigidity while allowing the tip some elasticity. Moreover the receiving part of the tube is provided with glands of problematic value.

In looking for further light upon the nature of this sperm transfer organ we turn to its development in the individual.

#### ONTOGENY OF STYLET

We find that in *Cambarus affinis* the first and second larval stages are externally alike, in both sexes, while the third shows the male openings on the fifth legs, or the female on the third legs. In the first stage, there are no abdominal appendages on the first somite and but a crowding of epidermal nuclei under the shell where the appendage will be. In the second stage, these appendages are slight papillae. These indifferent stages are fol-

lowed by the third, in which the external openings are differentiated but the appendages of the first somite are still simple papillae, alike in both sexes, unless they be longer in the male.

In the fourth stage, which is about 11 mm. long, the pleopods of the female still are simple papillae but little longer than in the third stage, while in the male they are long, simple spines, pointing toward one another and but slightly forward, as indicated in fig. 10 p. 127, Andrews, *Ontogeny of Annulus*, Biol. Bull., 1906.

The ventral face of the left spine or slightly specialized first pleopod, of a male 11 mm. long, is seen in fig. 16, magnified 430 in the camera sketch. This is from a larva killed July 1st, from late spring hatching. The organ is like a club; it is very simple, nearly cylindrical and very blunt. It is not jointed, although there is a faint groove marking off the base from what will be the neck and spiral.

On the base there is a slight ridge with depressions on the median side of it. Internally there are two muscles from the base into the sternum of the abdomen. The distal part of the appendage is slightly grooved along its ventral face, thus marking off an external from a median mass. In cross section, fig. 17, the shell is not very thick and beneath it is a well formed epidermis with large nuclei, from which connective tissue strands traverse the large blood space in which blood corpuscles float. This section shows the groove on the lower side. The appendage is articulated to a slightly elevated stump on the sternum that holds one of the articular muscles and part of the other and ends in an elliptical orifice into which the base of the stylet fits. This articulation is so oblique that the stylet lies down and cross-wise towards its fellow and is but little elevated or directed forward.

In the male of this stage, the openings on the fifth legs are short slits, not a third of the width of the above simple stylet, and to each slit there leads a strand of nuclei that represents the efferent duct.

In males of 15 to 18 mm., in the fifth stage, the stylet (fig. 18) is about 1 mm. long and is somewhat more specialized. The base is set off from the terminal part by a more pronounced fur-

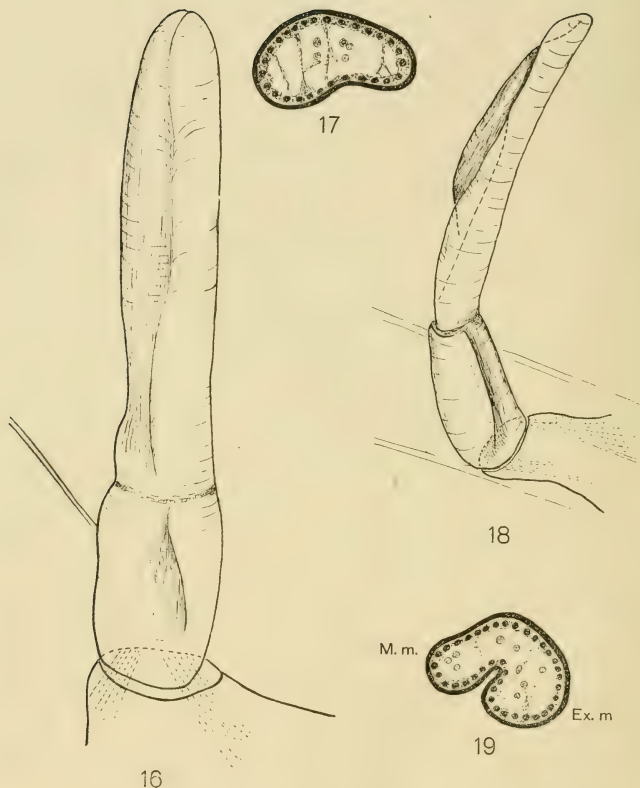


Fig. 16 Posterior face of left first stylet of male 11 mm. long. Enlarged 215 diameters.

Fig. 17 Cross section of the same stylet. *z. D.*

Fig. 18 Posterior face of left stylet of male 18 mm. long. Length of stylet 1 mm. *z. A.*

Fig. 19 Section of stylet of a male 12 or 15 mm. long. *z. D.*

row, but there is no movable joint. The organ is more pointed and the groove is very deep from the rising up of its sides. Thus in section fig. 19, the narrow median mass, (*M.m*) to the left, rises high up beyond the groove and the groove itself is a narrow space between the wide external and the narrow median masses. In the surface view, (fig. 18) the bottom of the groove is indicated by the broken line; it is already twisted so that the groove looks towards the median side along its proximal part and then for a short distance toward the observer, that is toward the ventral side, and finally at the tip toward the median side again. Where the groove is open ventrally the median mass is rising up as a protuberance that will form the spatula. As yet the canula is only the spoon-shaped end of the organ.

In a male 22 to 21 mm. long and probably in the sixth stage, (fig. 20) killed October 4th, we find the same stage as in other males of this size killed in July, this being an exceptional male that failed to grow as the average do to be nearly two inches long in October. Here the spatula is quite evident as a blunt rounded finger-like elevation that crosses over the groove. As shown by the dotted line the bottom of the groove is to the right of its mouth along the proximal part of its course and to the left along under the base of the spatula; that is, the sinuousness of the groove is exaggerated by the fact that the sides not only rise up but grow over the groove, the external mass overhanging toward the median line proximally and the median mass growing over away from the median line, distally. The base of the stylet now bears a few short acicular setae and is provided with three muscles at its attachment to the sternal elevation upon which it stands. By this time the stylets point forward under the thorax. The canula is now a short rounded blunt termination of the stylet in which the groove is no longer widely open but reduced to a slit by the up-growth of its walls.

In an autumnal male 38 mm. long, (fig. 21) the stylet has become much longer and more modeled but still shows the stiff joint between the base and the partly-formed neck. The few setae extend along the ridge of the base on to the proximal part of the external mass. The median mass sticks out abruptly at

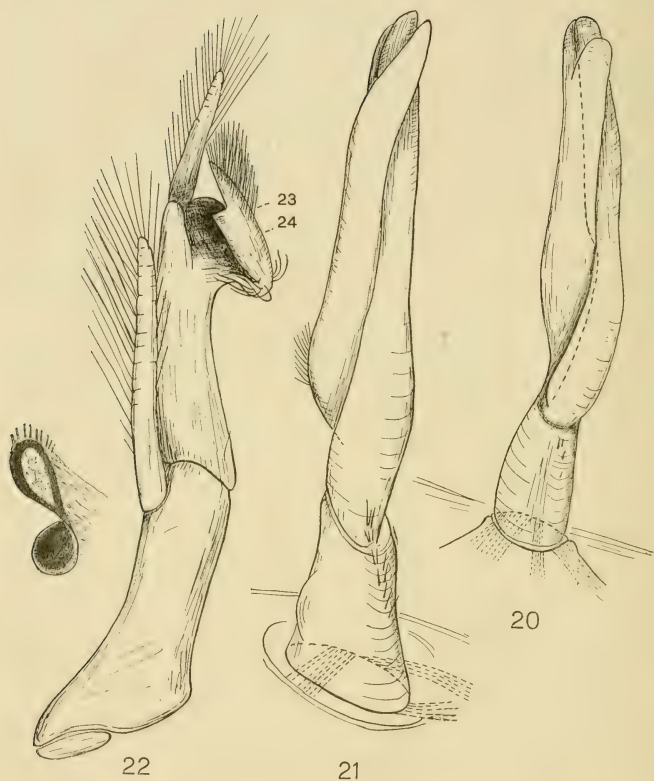


Fig. 20 Posterior face of left stylet of male 22 mm. long in October. *2. A.*

Fig. 21 Posterior face of left stylet of male 38 mm. long in October. Enlarged 25 diameters. *2. 90 mm. A.*

Fig. 22 Anterior face of left accessory stylet, somewhat turned to show part of the external face: a view between VII and VIII. *2. a<sub>0</sub>.* On the left an enlarged sectional view of the cup at the end of the radius and the wedge cut off.

the notch, or orifice, and bears a tuft of short setae. The spatula is long, flat and pointed. The canula is bluntly pointed and turned outward.

Later when the animal is 64 mm. long, the false joint of the stylet has disappeared and the tips become more sharp and long. Even before this size the males are known to conjugate, when about two inches long.

We thus find that the complex stylet of the adult starts from a slender papilla that becomes slightly flattened and grooved so as to form a very clumsy spoon with its depression rather more median than ventral. Then the sides of this groove grow up and make the groove into a cleft, which opens as before toward the median face proximally and distally; but along the middle of its course is forced to open ventrally and even externally by the overhanging growth of the median mass. The organ might be imitated by taking a long strip of clay with a slight length-wise groove on it and rolling the sides up over the groove, the median side tending to roll over outside the other. How the shelf from the external mass first grows out over the groove to cut off its inner part as a tube was not made out, but it is evidently a secondary specialization of the shell made by some special activity of the epidermis in a line near the bottom of the groove after the groove has become deep.

#### THE SECOND OR ACCESSORY STYLET

The accessory stylets (figs. v-viii) are evidently specializations of the common type of abdominal appendages, (fig. 26). They are elevated only when in use in conjugation; and at rest are carried forward under the thorax, horizontally, where they rest upon the first stylets and are closely packed in with them inside the special sternal groove of the male thorax.

Figs. v, vi, vii, viii, represent the left second stylet as seen from the ventral or posterior, the median, the anterior or dorsal, and the exterior faces, respectively. Like the unmodified pleopods this has a basal protopodite, an exopodite, an endopodite. The exopodite is a slender offset with setae, while the endopodite is

the complex large part of the appendage that bears a terminal flabelum and the remarkable side protuberance, found on no other limbs, which may be called the triangle.

Describing the entire stylet from the base outward, we see that the protopodite is chiefly a very strong flattened bony mass extending diagonally inward so that while the endopodite and exopodite are about parallel to the median line of the animal the protopodite forms an angle of  $45^\circ$  with it. This makes it possible for the endopodites of the two stylets to come together at the median line and for the endopodite of each side to lie upon the groove of the base of the first stylet, like a lance in its rest, although the bases of the two second stylets are fastened to the sternum of the second abdominal somite some distance from the median line. The protopodite is not entirely one-jointed but at its base is a soft membrane where it is joined to the sternum and in this are two large calcified plates, (figs. v and vi) besides two minute ones, (fig. vii) all of which together make a narrow basal section of the protopodite. Dissection shows there are muscles passing from this base of the protopodite into the sternum that may depress and elevate the appendage.

The protopodite is some 6 mm. long, 2 wide and  $1\frac{1}{2}$  thick. The exopodite is a slender filament some 9 mm. long and  $\frac{1}{2}$  mm. thick; a slightly flattened tapering cylinder set with long setae on external and median face. The setae are really plumose and together form a sparse brush. The exopodite is obscurely divided into some twenty segments. The basal 2 mm. is partly calcified, the rest membranous. It articulates freely with the outer distal corner of the protopodite so that it may be moved from the position of rest parallel to the endopodite, outward through  $90^\circ$  and swung back and forth some  $45^\circ$ . The tip of the exopodite often lies dorsally within the cavity or hollow of the triangle, and may have some use as a cleaning brush.

The endopodite is the stout calcified mass, roughly cylindrical but flattened from before back, some 9 mm. long on the median (fig. vi) and 7 mm. on the external face (fig. viii), and bearing at its distal end a flagellum on the external side and the flat triangle on the internal side. This bony mass is set on the protopodite

by a very stiff oblique joint at about  $45^\circ$  and allows of very little lateral and rotative motion. It may be forced outward and inward through but few degrees, its tip traveling only 4 mm. It may be twisted so that the triangle, from being almost concealed dorsal to the end of the bony mass (fig. v), may be turned outward a few degrees toward a horizontal position and present more of its median face, somewhat as in fig. vi. The movement is comparable to that of a stiff arm that should allow only a little side-wise movement and a very little twisting at the elbow with the end result that the triangle, or hand, at the end, accomplishes a little adjustment to the orifice of the first stylet. This is done as if by supination, though done by the above twist at the elbow.

The flagellum is the real termination of the endopodite; it is some 3 mm. long, 1 mm. wide and rapidly tapering, also flattened, being a long triangular terminal tip to the essentially flat endopodite. By the presence of white lateral areas in the otherwise membranous flagellum, it is obscurely divided into 9 or 12 joints. At the tip and along the sides it bears long plumose setae that are often sparse or worn off along the outer side. The flagellum springs from a socket in the bony shell of the wide end of the endopodite. The external angle of the edge of this socket, figs. v and viii, forms a hard protuberance at the end of a bony ridge (the Guide). The setae along the flagellum as well as those along the exopodite do not stand out horizontally, right and left, but slant ventrally, or posteriorly, (fig. viii).

The most novel and characteristic part of the second appendage of male crayfishes is the lateral outgrowth which we will call the triangle. It is a form of the Decapod *appendix masculina* of Boas. The triangle stands up dorsally so that at rest, it, with its fellow of the other side of the body, fits into the squarish cavity left between the two necks of the first stylets. It is not well seen normally from the ventral view, (fig. v) but it may be pulled outwards through  $90^\circ$  and then looks as in the median view (fig. vi). It is a flat triangular outgrowth, partly calcified and partly membranous. The edges are calcified and the centre membranous, so that the whole suggests a bent arm or wing with skin stretched across it. Each long side of the triangle is about 3 and

the shorter base about 2 mm. The bony rims of the triangle as seen in fig. VI may be called the humerus and the radio-ulna.

The distal free part of the apparatus, (figs. VI, VII) is a trihedral mass set with long plumose setae and might be likened to a sort of hand at the end of the fore-arm. We will call it the wedge from its appearance and use as seen in sections (fig. 9).

The humerus articulates at each end; proximally loosely with the side of the exodopodite mass, (fig. VI); distally at the elbow, firmly with the other firm edge of the triangle, the radio-ulna. On the external or concave face of the triangle, (fig. VIII) the humerus is not as well separable from the membranous part of the triangle, and between its proximal end and the bone of the main mass of the endopodite there is more or less expanse of membrane. On this outer face, (fig. VIII) we find that all the concave aspect of the triangle is membranous.

The humerus is wide and smooth and flat on the inner face, fig. VI, but on the outer face forms only a narrow edge to the membrane, fig. VIII.

The soft hollow face of the triangle in life is swollen with contained liquid. The soft area is not only the outer face of the triangular protuberance but also half of the dorsal face of the distal part of the main trunk of the endopodite.

The whole darkened area of fig. VIII might be compared to the soft inside of the palm of a hand and it is this which comes against the neck of the first stylet, in conjugation.

While the humerus is wider toward the base and slender at the elbow end, the radio-ulna is the reverse; that is, it begins narrow at the elbow and widens to the hand or terminal part. The radio-ulna is a thick plate-like mass that is not in the same plane as the humerus, but about  $45^\circ$  with it, so that it has the appearance of a scroll rolling in over the depressed membranous outer face of the triangle, (figs. VII, VIII). The radius part is the free rounded edge, (fig. VIII) and this ends abruptly opposite the base of the hand, which is back of it in the figure, while the ulna plate runs on continuously in the background of this figure and passes imperceptibly into the hand, or wedge, (fig. VII).

The radius stands free, away from the membrane, as a rounded

bony ridge much thicker than the ulna plate from which it is faintly marked off by a suture. Thus in sections (fig. 8), the radius looks like a head on a slender neck. The abrupt termination of the radius is very like the elbow end of the human radius, a shallow cup. The actual cup is made by clear horny matter of considerable thickness and is prolonged as a horny sharp ridge all along the radial edge of the pyramidal wedge. The head of the radius stands out as wider than the neck (fig. VII).

The ulna is but a vaguely defined thick area of the general shell and it continues as the hand or wedge, which is, next to the head of the radius, the most peculiar part of the triangle. This wedge is a hard horny pyramid of three faces. One is rounded and setose, two flat, meeting at a sharp edge, (see small sketch, fig. 22). Its exposed rounded face (figs. VI, VII) is set with a dense brush of plumose setae. The external or ulnar face (fig. VII) is smooth bone, bearing setae along its right edge and ending, to the left, in the sharp horny ridge that runs up from the head of the radius and is shown as a dark shade in fig. V. The concealed innermost face is bony and contains orange pigment; along its left edge it bears setae (fig. VIII), and its right edge is the sharp horny membrane that runs up from the head of the radius. In the union of this face with the soft membrane of the concavity of the triangle there is a bony articular plate.

The photographs do not represent one feature of the triangle and that is the small tuft of some five or six, or so, very wiry bent plumose setae that spring from the elbow of the triangle and, for the most part, curve so as to lie down close to the soft membrane. These setae are roughly shown in fig. 22 at the elbow. This also gives in the side sketch, an end view of the head of the radius as seen when the base of the wedge was cut off and the stump of the ulna and free end of the radius viewed from the face where the wedge had been. This is intended to show the head of the radius as a rounded saucer with flat bottom, not deep, but with flaring and rounded sides that form a rim thicker than the neck of the radius below. The cut off setae in this figure are the bases of those on the union of ulna and wedge, just above the level of the line 23 in the main fig. 22.

## INTERNAL ANATOMY OF THE SECOND STYLET

Dissections and sections showed the presence of the same general structures as in the case of the first stylet, with the important difference that the special glands of the tube of the first stylet are absent and on the other hand the intrinsic muscles that are absent in the first are well developed in the second stylet. The muscles are arranged as in the younger stages (figs. 27, 28). Besides the three muscles at the base that pass into the sternum of the second abdominal somite a very short distance there are long muscular strands within the stylet itself.

The protopodite springs from a considerable elevation of the sternum and in the adult two muscles were found within this elevated articular region. Pulling one tended to depress the stylet into its position of rest while the smaller muscle was thought to be probably concerned with the erection of the stylet. Pulling all the basal muscles made the stylet not only lie down but also move toward the median line, which would enable it to fit in nicely with the first stylet. Some of these extrinsic muscles extend a distance into the protopodite itself, to be attached to the shell. There are also long strands arising from the shell of the protopodite and running to the exopodite and the endopodite. Those of the exopodite seem associated with the basal muscles, so that pulling the muscle in the sternum made slight twitching movements of the exopodite, simulating those seen during conjugation, which may thus be caused by contractions of the muscles that hold the entire appendage in position. Pulling the muscles that are in the distal part of the protopodite made both exopodite and endopodite move dorsally and also away from the median plane.

The muscles that move the exopodite are better developed than those of the endopodite. Within the exopodite there is a long intrinsic muscle that would seem fit to bend the slender filament slightly. Inside the endopodite, beside the slight muscles of the base concerned with the movement upon the protopodite, there are in the adult two slight threads that represent the muscle seen in early stages (figs. 27, 28) passing from the terminal flagellum down

into the region whence springs the triangle. These muscles are seen in the sections of the triangle (figs. 23, 24) as two black dots.

These sections, with those in figs. 8, 9, show the anatomy of the triangle. Fig. 23 is a section along the line 23 of fig. 22. The great thickness of the calcified shell is shown by the black mass. The membranous parts are shown by the thin black, as to the left in fig. 24. The cavity within is blood space traversed by connective tissue strands and faced by epidermis against the shell. In

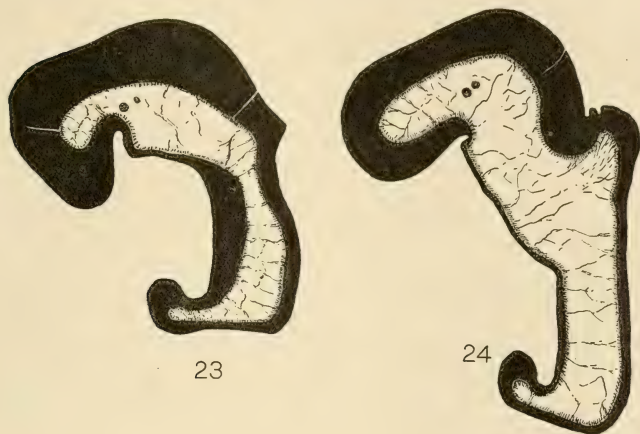


Fig. 23 Cross section of the triangle on the level 23 of fig. 22. *z.* 90 mm. *A.*

Fig. 24 Cross section of the same about the level 24 of fig. 22. *z.* 90 mm. *A.*

fig. 23 the triangle and the distal part of the protopodite are cut across with the hollow face to the left. The dense shell mass to the left above is the guide ridge, (fig. VIII) which somewhat overhangs the cavity of the triangle and bears on its median face some setae, (fig. VI), which are connected at the root with the epidermis by the long canals of which one is seen to the left (fig. 23) penetrating the shell. Opposite this on the median face of the endopodite there are also a few setae which do not appear in the

photograph (fig. v) but present one of their canals in the shell of fig. 23, to the right. In contrast to the excessive thickness of the shell of this main stem of the endopodite, the triangle, as represented by the lower part of this section, is relatively thin shelled. The radius is the thick knob in the lower left corner. The shell to the right is the ulna, the thick mass against the concavity of the triangle is in reality more membranous than calcified, but as yet thick. But further toward the elbow (fig. 24) along the line 24, (fig. 22), the corresponding region is a thin membrane reaching from the neck of the radius across to the thick guide ridge. In reality the elbow stands out more as in fig. viii so that the width of the section 24 is much greater than fig. 23. Fig. 24 shows clearly, on the right, the hinge-like line of demarcation between the outstanding triangle and the main stem of the endopodite, being in fact cut at the edge of the proximal articulation of the humerus (fig. vi), where there is a sudden change in level in passing from the humerus to the main stem. In sections 23 and 24, the small black dots above within the connective tissue, are the museles that run up into the flagellum, much as in fig. 28.

Section 8 shows the radius standing out from the flat triangle with the thick mass of the humerus above in the figure, while fig. 9 shows the thick end of the endopodite above and in the groove of the first stylet the cut off wedge, as will be described below in considering the adjustments of the first and second stylets during conjugation.

#### ONTOGENY OF THE ACCESSORY, OR SECOND, STYLET

Between the individual development of the first and the second stylets there is this important difference that while the first never at any time looks like one of the ordinary pleopods but is of late appearance and is also a dwarfed, specialized, or reduced appendage from the first, the second appendage is present as soon as the others are and is at first like the ordinary appendage and becomes specialized by the addition of an outgrowth and not by the loss of parts.

The pleopods of the second, third, fourth and fifth somites of both males and females are represented at the time of hatching and all alike have the appearance seen in fig. 26 which is magnified 75 diameters and represents the anterior face of the third left pleopod of a male 18 mm., in July, when in the fifth larval stage. The pleopod is flat and translucent; the endopodite (*En.*) is longer than the exopodite (*Ex.*) and both are fringed by long setae that are really plumes, though not so figured. Both endopodite and exopodite are obscurely jointed and the protopodite has a short annular segment as well as a long main segment. Through the thin shell may be seen the muscles, represented by the dotted lines. At the base are three large and one minute muscles; two of the main three are posterior and one anterior, and apparently the movement of the entire appendage would be a more powerful backward swing and weak forward recovery, as in swimming. Within the main segment of the protopodite are three long muscles that would seem to aid in bending the appendage at its base, while distally there are two muscles which both go to the exopodite to move it. The endopodite is left with only intrinsic muscles to move it at its base and with a long branched muscle that can act only to bend the endopodite itself. The exopodite has also intrinsic muscles at its base as well as the muscles of the protopodite to move it. There is likewise a long branched muscle to bend the exopodite.

In the early stages the second appendage of the male is quite like this third pleopod, but in a male of 21 mm. (probably in the same larval stage as the male having the third appendage shown in fig. 26) we find the pleopod of the second somite modified as in fig. 27, that is, there has been added to it the excrecence seen on the median side of the endopodite. This is to become the triangle or *appendix masculina* of the adult.

The first discovered trace of this outgrowth was seen in a larva of the fourth stage, 11 mm. long, in July. This first beginning of the triangle is the slight elevation (*x*) seen in fig. 25, on the side of the endopodite. This figure represents only that part of the endopodite which is not well jointed and forms a sort of base beyond which is the more flabelliform distal part, (fig. 26). It

will be noted that the row of plumes on the right, or median side of the endopodite (fig. 25), is interrupted distally so that there is a blank space where one would expect one or two setae, and in this space there protrudes to the right a rounded elevation. The position of this slight elevation with reference to the muscles leaves

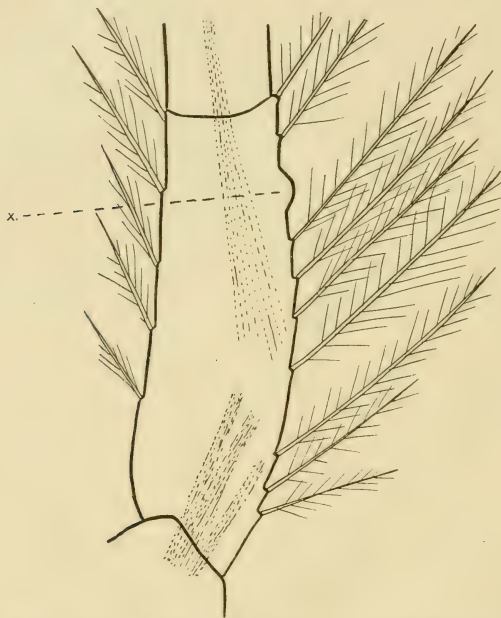


Fig. 25 Posterior view of basal part of the endopodite of the accessory stylet of a male 11 mm. long. Enlarged 215 diameters.

no doubt that it is the same thing as the larger elevation of the next larval stage (fig. 27). In the preparation the epidermis, not here shown, grew out to form this elevation as a hollow outgrowth, leaving no question as to the possible artificial nature of the bulging of the cuticle shown in fig. 25.

In the fifth larval stage (fig. 27) the protopodite has become wider and stouter and the basal part of the endopodite is much expanded distally where the protuberance arises from it. The

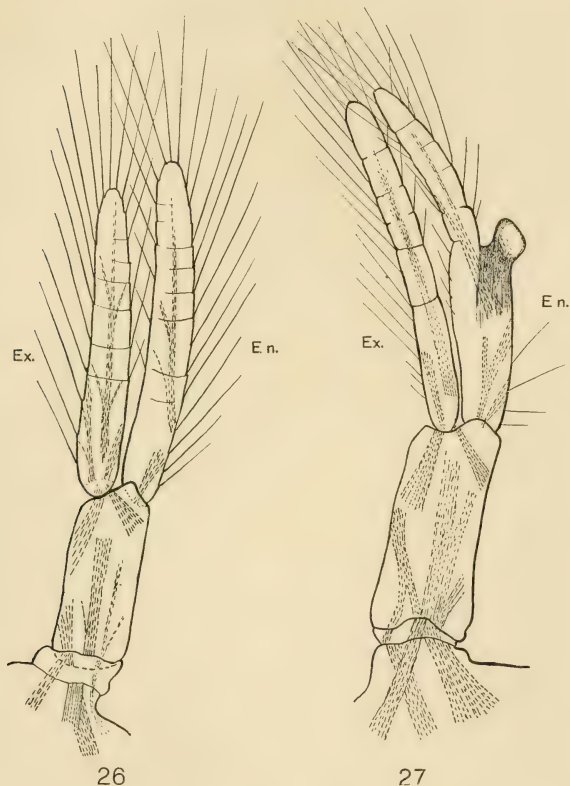


Fig. 26. Anterior face of third left pleopod of a male 18 mm. long. 2. *D.*

Fig. 27. Anterior face of left accessory stylet of male 21 mm. long. Enlarged 75 diameters.

result is that the exopodite begins to take on that relative insignificance in size, characteristic in the adult accessory stylet.

The new growth on the median side of the basal part of the endopodite (fig. 27), is a sort of knob set on a neck and inclined at about  $45^{\circ}$  to the axis of the endopodite. Its form is not spherical but rather more that of a short cylinder on a slightly shorter neck. The long axis of the cylinder and of the neck is at an angle of  $45^{\circ}$  degrees to the side of the endopodite. Not only this protruding knob must be reckoned as part of the future triangle but also the neighboring widened area of the endopodite which is depressed as indicated in the shadow in fig. 27 and which will be the depressed anterior face of the future triangle. In fact this depression is accentuated by the position of the knob, which not only stands out as represented in the figure but also rises up toward the observer; that is, anteriorly away from the general plane of the endopodite. The base of the flabelliform distal part of the endopodite is continued on to the external distal corner of the basal region of the endopodite as a ridge standing up above the depressed area, and forming what will be the guide ridge of the perfected organ.

In a small male, 38 mm. long, in October, the second pleopod had advanced to the state of perfection shown in fig. 28, which is an external view of a left accessory stylet, which was about three times as long as the one shown in fig. 27. The muscles in the protopodite remain as before, though not so well seen from this point of view, and the same is true of the endopodite and the exopodite. The protopodite and the exopodite have grown so large and massive that the slender exopodite is much subordinated. The great increase in the basal part of the endopodite, along with the enlargement and specialization of the triangle, leaves the plumose terminal part of the endopodite as a slender palp-like remnant of the original end of the endopodite. The triangle is now so much longer at its free edge than at its attached part that it has the adult triangular form when seen from the median face; or more explicitly, the obliquely set cylindrical knob of fig. 27 has grown so much longer at its free edge than at its attachment that the length between its ends about equals the distance of the proximal end or elbow from the main mass of the endopodite, which

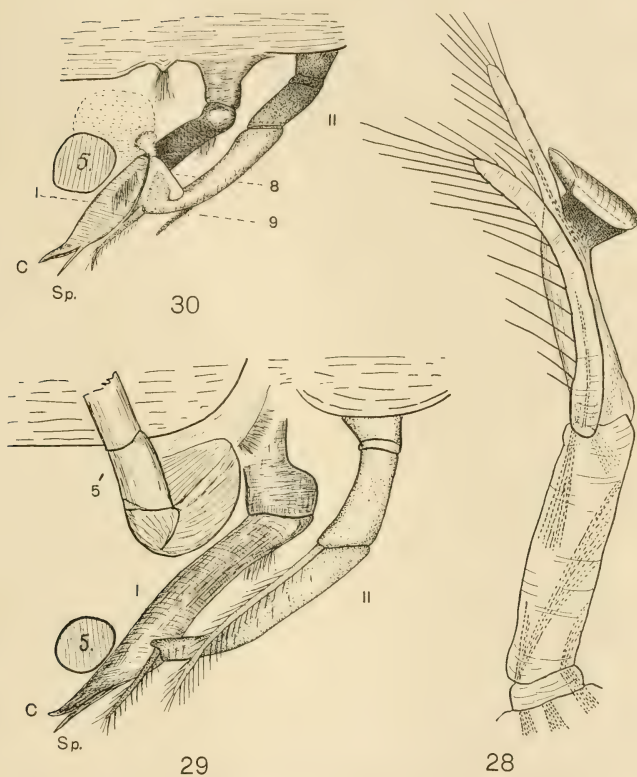


Fig. 28 External face of left accessory stylet of male 38 mm. long in October, enlarged 25 diameters.

Fig. 29 External view of the united first and second stylets of the left side of an adult male, 110 mm. long. 2. 90 mm.  $a_0$ .

Fig. 30 View of the median face of the same. 2. 90 mm.  $a_0$ .

In figs. 29, 30, 31:

I = 1st stylet

II = 2nd stylet

5 = crossed fifth leg seen in section

5' = other fifth leg not crossed.

C = canula

Sp = spatula.

gives the wide scalene triangle as seen from the median side (fig. VI). The proximal elongation of the cylinder makes the elbow of the triangle, while the distal elongation has made the pyramid or wedge that runs up toward the flagellum of the endopodite. As yet no setae were seen on the wedge. The triangle, however, is not merely a flat plate that grows out diagonally, but from the first it is thick through in the anterior-posterior direction, thus producing the cylindrical edge seen in fig. 27, where the thick edge is restricted and marked off by a less thick neck; moreover the thickening of the cylinder is toward the anterior face. By the stage shown in fig. 28 there is great thickening toward the external face. Moreover the external free edge of this thickened cylinder is now itself thickened as a ridge hanging out from the ventral rim over the depressed area as indicated by the broken line in fig. 28. This rounded thick edge is the future radius. (Compare figs. 28 and VIII.) From this state it is an easy transition to the more sculptured form of the appendage seen in adults.

The second pleopods of the male thus owe their special structure to a gradual emphasis of the endopodite and protopodite with the addition of an outgrowth peculiar to these appendages, the triangle. The triangle at first is a mere blister on the median side of the endopodite but soon becomes an oblique plate that is surmounted by a thickening. The plate grows anteriorly and the thickening of its free edge becomes longer than the base of the plate, with a resulting triangular form as seen from the median face. The thick ridge grows out externally and this extension itself acquires a thickened rim, posteriorly, which is the radius.

The triangle is thus a triangle only as seen from the median face of the pleopod, in its entirety the triangle is a curved object like a half open hand, and as such is capable of being applied to the rounded surface of the first stylet. It is made of a cylinder obliquely set along the edge of a plate and curving over it, like fingers over the palm. A slip of paper if cut of angular form and bent twice at right angles may be made to represent the stylet.

## USE OF STYLETS IN CONJUGATION

The way in which the various parts of the stylets are used in the process of conjugation and sperm transfer has been found out partly by direct observation, partly by experiment, and partly by more indirect inferences that still leave some questions unanswered.

The phenomena of conjugation in general have been described elsewhere (5) and we will here consider chiefly the use of the stylets. There is a stage in the early part of conjugation, where the male has seized the female and clasped all her claws, when he rises up away from her sufficiently to allow the pleopods to swing back and forth. In this swinging the long stiff stylets and accessory stylets take part and then are soon locked together, after which the stylets are held by the crossed fifth leg so that henceforth they make a rigid mass which cannot be folded down against the thorax again by any pressure until that fifth leg is removed. The process of locking together of the stylets is as follows:

The swinging of the pleopods is caused by their basal muscles; and likewise the muscles in the bases of the stylets move them slightly backward, or erect them, and forward, or depress them. While both first and second generally move together and right and left alike, they have been seen to move independently. By a special movement of the second stylets they are clasped against the first in such a way that the triangle is applied to the neck of the first stylet. By arching the abdomen, cat-like, the second stylet is drawn up dorsally along the first, and then, by partial relaxation of the arch of abdomen, the second is shoved distally along the first, while held tight against it; the result is that the wedge glides along in the groove of the stylet and the radius enters into the inner tubule through the flaring orifice and is shoved in so far that it remains fast. In sections (fig. 8) it is seen that radius fits into the groove as in a socket and, all the walls being thick and solid, the radius cannot be forced out again without running it back along the orifice. The fact is that the locking is very firm and when one tries to pull the second stylet backward the first is dragged with it and only by pulling the second dorsally toward

the base of the first can one separate the two, as by that means the radius is brought to the orifice out of which it readily passes.

When the two stylets have been erected by their own erector muscles and locked together by their muscular movements which lead to this mechanical fastening of the edge of the triangle within the groove, they form one organ, physiologically, which is to transfer the sperm without any further muscular activity within it.

The appearance of the two locked organs is indicated in the somewhat diagrammatic sketches 29, 30. In 29 the external view of the left stylets and part of the fifth thoracic legs is shown. The second stylet, to the right of the figure shows the solid tip region of the endopodite applied closely against the most protuberant part of the posterior face of the spiral of the first stylet, while the terminal flabellum runs along parallel to the canula and spatula. In fact the tip of the bony endopodite seems to overlap the contours of the spiral and this is due to the soft nature of the depressed region of the median face of the end of the endopodite as is seen in fig. VIII. The guide ridge is the part seen external to the spiral in fig. 29, while the soft surface is squeezed against the rounded face of the spiral and the triangle is applied close against the median face of the spiral so that it can be seen only from the median view.

Turning to the median view we see, (fig. 30) the triangle lying over the neck and extending out along the groove. The elbow of the triangle lies over the orifice. The radial edge of the triangle conforms with the obliquity of the groove since both the wedge and the radius are firmly inserted in the groove.

Figures 29 and 30, show the supporting fifth leg in section, as a rounded cross-hatched area. It will prevent the locked stylets from being shoved forward, or closed up against the sternum anteriorly. It is also obvious that the movement backward toward a vertical position will be hindered, not only by the inclination and rigidity of the basal joint of the first stylet, but by a like joining of the base of the second stylet, since one cannot move back without the other, for the radius and wedge will go no further

toward tip of groove. The second forms a mechanical brace tending to hold the first from going backward.

In order to separate the two the second must move toward the animal and glide along the first till free from it. And this motion is actually seen. The locking is not always done without trial and may be broken and renewed during conjugation, so that we often see two positions of the stylets, that of perfect locking,

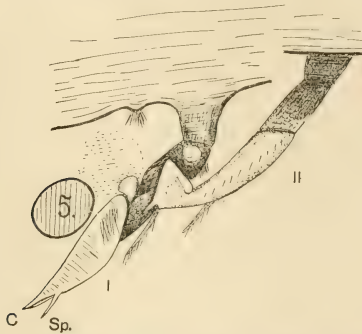


Fig. 31 Same view when the accessory is drawn back into position of recession showing the papilla at the mouth of the groove.

as in figs. 29 and 30 when the triangle is most advanced toward the tip of the spiral, and a preliminary and alternate position of recession when the triangle is applied against the base of the first stylet proximal to the orifice. This position of recession is shown in fig. 31. The triangle goes as far toward the basal end of the first stylet as possible, till stopped by the knob on the base (fig. II). In this recession the orifice with the papilla meeting it, is exposed and the ventral lip is seen.

It should be borne in mind that the back and forth play of the triangle on the first stylet is limited not only by the knob basally and the narrowness of the groove that prevents the radius from going into it dorsally beyond the position of figure 30, but it is limited laterally by the fact that the triangles of the two sides

of the body are in contact and are held together by being placed in the squarish hole between the necks of the first two stylets.

The two triangles play back and forth like two hands with bent fingers, back to back, in a narrow space between the first stylets and, like hands, each runs its palm or soft flat surface along the median constricted part of the first stylet and the firm guiding ridge—its thumb, as it were—along the external face of the stylet (fig. 29). In one case from 3 to 4 seconds were taken to glide the triangles back from the normal position to the recession (fig. 31); there they remained four or five seconds and advanced strongly in two seconds. Another recession took 12 seconds, but the advance occupied 2 seconds.

If we imagine figure v applied to I, VI to II and VII to III, VIII to IV, we will appreciate how nicely all the surfaces adjust themselves. The oblique ridge of the external mass of fig. I is overlaid by the soft depressed area, (figs. VIII, 22) so that the thumb-like guide shows external to the ridge as in fig. 29.

In life the two sets of appendages, right and left, are so closely applied together that the median face of neither can be seen, directly, without mutilation experiments on one side, but the presence of the guide ridge along the external face of the spiral (fig. 29) enables one to judge where the triangle must be at any stage of advance or recession, a matter of importance in deciding as to its use in sperm transfer.

That an application of the second, or accessory stylet, to the first is necessary for the completion of normal conjugation and the filling of the sperm pocket by transferred sperm, was determined not only by the above facts of structure and use but by the following experiments. The instincts of the male are so strong that, when in the process of conjugation the second stylet on one side was cut off, there was no immediate visible effect, except the escape of some blood from the stump of the appendage. And when on the next day all the stylets, both first and second, were cut off, the male seized and turned a female and carried the conjugation as far as possible in the absence of the organs of transfer. The instincts thus go on without the means of carrying them to completion.

It was then easy to get males to begin conjugation when the accessory stylets had been removed from both sides. Three such males made conjugation experiments with several females, successively, but in no case was there an evidence that the annulus had been filled by these mutilated males, through in one case the union lasted for eight and one-half hours. In these attempted conjugations it was not evident how the absence of the second stylet prevented perfect sperm transfer. In one case the male let fall three or four sperm masses, or pseudo-spermatophores, about 1 mm. long on the telson of the female but it was not determined how this happened. Apparently this was from failure to have a close union at the orifice, which would lead one to think the failure due to absence of the triangle that normally holds the papilla tight to the orifice. But the failure may have been due to the absence of piston like movements of the radius. More experiments should show what the uses of the different parts of the triangle really are.

#### HOW THE SPERM IS FORCED ALONG THE TUBULE OF THE STYLET

The adjustment of the papillæ, whose anatomy has been described, to the stylets must now be considered in order to appreciate the final use of the stylet.

As seen in fig. 1, the papilla juts out toward the median plane so far that it can be placed across the narrowest part of the first stylet where the notch is (fig. III); that is across the dorsal face of the first stylet. But its tip turns abruptly inward far enough to reach along the median face (fig. II) as far as the orifice, into which its tip fits. In figs. 30, 31, this position of the papilla is crudely represented; in reality the tensely swollen translucent spout is very nicely applied to the rounded faces of the entrance to the groove. The papilla is seen in this position when the triangle is receded (fig. 31) and in the advance of the triangle its tip becomes concealed, but it doubtless remains as before.

Returning to the actions of the combined stylets which embrace the papillæ we note certain 'tamping' movements. Besides the advance and recession of the second stylet along the first, the first and second together when locked, are seen to execute quick jerks

that carry the tips of the first back and forth a part of a millimeter only. When the tips of the stylets have gained entrance into the annulus, these thrusts may serve to introduce the tip farther into its cavity. As in the movements of recession the force here must be exerted by the muscles of the abdomen, as the stylets themselves have no telescopic power; and actual twitching of the anterior part of the abdomen were seen.

#### SPERM EMISSION AND CONDUCTION

In normal conjugation nothing is seen of the sperm so that its transfer from the deferent duct to the cavity of the annulus is a matter of inference. The papilla is applied to the orifice of the tube of the first stylet so that it may discharge into it and sections show the tube full of sperm, (figs. 14, 15); moreover in some abnormal cases the sperm is seen to issue from the tip of the canula into the water, and, as the tip of the canula is normally inside the sperm pocket, it is evident that the sperm must pass along the stylet from the papilla. The force that propels the sperm is no doubt muscular contraction, but it is not clear at first what muscles are concerned; there are none within the first stylet which acts merely as a passive tube.

From such figures as 2, it is evident that the deferent duct has powerful transverse muscles that could squeeze out the sperm with force and this seems the main if not only motive force to carry the sperm through the papilla and all along the tube of the stylet into the annulus.

The force necessary to propel the liquid sperm through a tube that is only some 20 to 40  $\mu$  in diameter (figs. 13, 15) is great and attempts to force india ink through the tubule of the stylet with a small hypodermic syringe failed. When the specially ground canula was inserted into the orifice, while the radius was engaged in the tubule, no ink could be forced out of the tip of the stylet. It was inferred that the radius blocked the way, as it fits in so as to nearly occlude the lumen (fig. 8), but the same failure was met with when the triangle was removed from the stylet, but then the ink jetted out along the proximal part of the groove where the

triangle had been. Apparently the wedge of the triangle is well fitted to hold the liquid in the tubule since it fills up the groove external to it (fig. 9), where the sides of the groove are not as close together as they are distally (fig. 10), which is beyond the wedge. When the ink had been introduced into the tubule and not forced out of the tip of the stylet the triangle was applied to the stylet and the radius worked back and forth like a piston in the tubule with the result that some of the ink issued from the tip of the canula of the stylet.

This suggested that the radius might act like a piston in normal sperm transfer and thus propel the sperm from the papilla along the tubule to the annulus. We also saw that when a pair was separated in conjugation the sperm that issued from the tip of the canula of the stylet was mixed with bubbles of air when held out of the water, which suggested some action at the base of the tubule (at the orifice) to draw the air into the tubule. However, this might be movements of the triangle or simply failure of the triangle to hold a tight joint around the tip of the papilla and orifice, for thus air could be drawn in by the stream of sperm advancing, driven by pressure of the muscles of the deferent duct. When the radius was inserted into the orifice and shoved along in the tubule, sperm was forced out of the tip of the canula, which seemed to demonstrate the ability of the radius to act as a propelling piston.

We failed to detect any such piston motions during conjugation, but they would be of very slight extent and not readily observed. The movements of advance and recession described above are of a much grosser magnitude than the piston movements that might be supposed to take place. The movements 31, 30 are only for getting right adjustment of the enveloping triangle over the papilla tip and the entrance of the radius into the tubule so that the hand-like triangle may make such tight binding of the papilla to the orifice that no sperm escapes or comes into contact with the water. Yet the piston may then presumably be in position to advance or recede a little. When we thrust the triangle strongly so far along the stylet that the elbow was at the orifice, (fig. 30), the triangle tended to spring slowly back out of the groove

till only half of the length of the radius remained in the groove, owing apparently to the elastic side walls of the groove shoving against the wedge (figs. 9, 10) as these walls are the closer together toward the tip of the stylet.

By this mechanical means the piston might tend to recede, while the movements of the muscles of the abdomen might make the entire second stylet advance enough to shove the piston along the groove again. We can easily pump the radius back and forth in the groove by moving the whole second stylet. The muscles of the abdomen make the slight twitching back and forth jerks of both first and second stylets above mentioned as tamping movements. Now after the first stylet, with the second locked to it, is introduced into the cavity of the annulus as far as possible, these movements of tamping, if they be continued, could not advance the first stylet but may push the second further along the first and so cause the piston to act on the sperm. The dish-like head of the end of the radius (fig. 22) receives explanation upon the assumption that it is useful in shoving the sperm along in the tubule, in fact, the solid bone-like piston with horny cupped tip provided with elastic flaring edge seems a remarkably well made apparatus for pushing liquid along in a tube that it fits so well.

Some such piston movements might be expected from the statements of Coste (C. R. 46, 1858), that Gerbe in his laboratory saw the male *Astacus* apply the foliaceous part of the second stylet to the first stylet and by reiterated back and forth motions during the passage of sperm, keep as he thought, the trough of the first stylet free from sperm that might harden there else. Schilling, states that the second stylet is used to push the spermatophores out of the first stylet.<sup>1</sup>

The groove and its concealed inner part that forms the tubule are of course open to the water and if the sperm is to pass free from contact with the water to the cavity of the annulus the assumed piston movements of the radius may serve to clean out the tubule and fill it with harmless secretions. The source of such secretions may be surmised to be the glands in the tip of the pap-

<sup>1</sup> As reported by Ortmann in Bronn's Klassen und Ordnungen.

illa (fig. 2) or those along the tubule itself (fig. 8). Possibly this preparatory action of the radius is all that it has to fulfill and that the pressure of the muscle of the efferent duct is all sufficient to cause the sperm to run through the length of the stylet. In connection with this question we have to bear in mind that the sperm is in some way freed from its envelope of secretion made in the efferent duct before it is laid away inside the sperm pocket where it exists pure (1).

This separation of sperm from enveloping secretion takes place in the tubule of the stylet. In the proximal part of the tubule the secretion of the deferent duct (fig. 2), is still all around the strand of sperm (fig. 14), but distally the sperm is almost pure inside the tubule (fig. 15).

We found also that in one case a male, fallen on the side while still holding a female, had the stylets only partly erected so that they were free in the water and from the tip of each canula a very fine stream of sperm, finer than the tip of the spatula, issued slowly and coiled up in a small mass. From one canula the sperm then slowly sank in ten minutes down in the still water as a fine thread with a coil at the tip. Another male showed faint sperm jelly on the tip of the flagellum of the endopodite of the second stylet and this was pure sperm becoming modified by the water; there was no secretion.

There are however besides these escapes of pure sperm, escapes of sperm inside of secretions that resemble spermatophores. In a male, in which the triangle was in the position of recession, (fig 31), there were such white sperm threads,  $\frac{1}{2}$  to 1 mm. long, about the orifice of the groove. The pseudo-spermatophores that in abnormal or interrupted conjugations were sometimes seen were soft, paste-like tubes containing a central mass of sperm. The short pieces of tube stick by their ends to the inside of a pipette used to pick them up and to the shell of the crayfish on which they fall.

The wall of these tubes is a very thin layer of secretion which is vesiculate and stringy like dough and can be drawn out into clear threads with minute droplets along them. These would seem to be not normal spermatophores, which in *Astacus* have

thick walls, but only rods of sperm enveloped in some slight secretion from the deferent duct, or possibly that of the papilla or of the glands of the spiral. The thin walls of these tubes break open, hernia like, and sperms ooze out.

The separation of the sperm from the secretion of the deferent duct may be due merely to the diminution in diameter of the tubule; the pressure of the duct would drive the central part of the current faster than the envelope and thus the central sperm might flow out of the very narrow tip of the canula and leave the envelope of secretion behind in the wider parts of the tubule. Finally, when enough sperm had passed along to fill the annulus, the enveloping secretion might be forced out and this would make that wax-like mass that fills the external parts of the annulus and projects in excess from its mouth as the so-called sperm plug. Possibly again the piston movements of the radius might come into play to clean out the secretion from the stylet tubule and ram it into the annulus. In fact in the last stages of conjugation of one pair slow and repeated movements of advance and recession of the triangle were seen which may be interpreted as concerned with plug making.

The use of the glands of the spiral is not known. Possibly their secretion cleanses the surfaces to be used in sperm transfer and aids in keeping water from the sperm. Possibly the secretion may help the enveloping secretion of the deferent duct to adhere to the walls of the tubule of the spiral and thus hold it back till the sperm has passed on into the annulus.

#### THE RIGHT AND LEFT DUPLICATION OF STYLETS

The striking fact of the exact duplication of both first and second stylets right and left suggests questions as to the use of right and left in conjugation. Are both sides used at each conjugation?

Again the remarkable dimorphism of the females of *C. affinis* and of probably all other species of that genus, which expresses itself in the occurrence of females with the vestibule of the sperm pocket opening a little to the right of the middle line and of females with the pocket opening to the left, so that the symmetry

of the two is reversed, raises the question as to whether the males are adjusted, in habit, to these two kinds of females, so as to use the right set of sperm transfer organs for a left-handed female and vice versa, or not.

The crucial experiments to determine whether males actually use the one stylet for right and the other for left-handed females have not yet been made. However, some facts and considerations make it improbable that a male is obliged to do so and indicate that a male may adjust his stylets so as to use either right or left on any form of female annulus, leaving the question still open as to what is the normal habit of the males with reference to the two forms of females.

In the first place we found that though the two first stylets seem to be in the annulus they are never both firmly inserted. One is fixed firmly by its tip while the other may be drawn away by a pair of forceps. Moreover the one that is inserted has its tip some  $\frac{3}{4}$  to  $1\frac{1}{2}$  mm. in advance of the other and its base is locked against the base of the other, diagonally, the abdomen being advanced more on one side than the other.

Observations showed that not only were there cases of the right stylet in the left annulus but of right stylet in the right annulus and of left stylet in left annulus and of left stylet in right annulus. Whether in these cases the sperm was actually transferred was, unfortunately, not made out. It is possible that a male may insert one stylet and afterwards the other till finally the actual sperm transfer takes place with some more definite reference to the symmetry of the annulus than the above observations would indicate.

That there is any alteration in the advance of the stylets was not made out, but there is often an alteration in the use of the fifth leg, right and left. At any one time many males will be found with the left and others with the right leg crossed, but continuous observations show that the male will change from right to left in difficult cases especially, till a better adjustment is obtained.

It was at first thought that there was a relation between the fifth leg and the advance and use of the first stylet so that these were on the same side, that is, the stylet being advanced by the

use of the leg of that side of the body, but cases were recorded in which the advanced stylet was on the opposite side from the crossed leg. Males crossed the right leg with either right or left stylet advanced and males crossed the left leg with either right, or left stylet advanced. Here again there is the possible objection that the condition observed was not permanent or the one employed in actual sperm transfer. More minute observation of several normal cases are necessary.

One good case seems, however, rather conclusive. In this a male, in November, crossed the left fifth and advanced the left stylet, but after an hour of attempts to enter the annulus, crossed the right fifth and five hours later the right stylet was one mm. in advance of the other and the female had a sperm plug in a right annulus. Here the leg and stylet used did coincide, but the annulus was not the one to be expected.

Again in some conjugates killed by boiling while united it was found that in one a right stylet was advanced to a right annulus and in others a left stylet to a left and to a right annulus.

As far as the evidence goes it gives the impression that the male is free to use either right or left stylet with either right or left fifth legs till successful in getting some one tip of the stylets into the vestibule of the annulus, which may be a right or a left one, indifferently. Yet future observations may show that the lines of least resistance are for the male to use the left stylet for the right-handed female, and the reverse, and that this actually takes place, in nature as the normal, though we doubt if it be at all necessary. Observations show that both papillæ are ready to discharge sperm at the same time and it should be determined by experiment whether the male uses both right and left sets of sperm transfer organs, alternately, at each conjugation or not.

When the first and the second stylets were cut off from one side of some sixteen males and, either at once or some weeks after, these males were given females, the unexpected result followed that in spite of many repeated attempts, one lasting nine hours, the numerous conjugations of these unilaterally mutilated males did not result in any clear cases of successful sperm transfer. In

many cases the annuli of the females were artificially cleared out so that any new plugs would have been seen.

Among these cases there were males that alternately used the fifth left and right legs in crossing, though some had only the left series of stylets and others the right; the leg being crossed on the side where there was no stylet and on the side where there was a stylet. And these same cases were attempting conjugation with females that were of both kinds, right and left forms, so that there was no agreement between the kind of annulus and the fifth leg used.

In only one case was there any sperm seen and this was seen twice in successive conjugations of the same male that seems to have been peculiar. This sperm lay in pseudo spermatophores, 8 mm. long, upon the telson of the female under the left stylet, and probably escaped from some imperfection of the closure of the triangle.

While it was not found out why there was this apparent inability to complete sperm transfer while the stylets of one side were missing it is thought that this is not due to the need of using sperm from both sides of the body at each conjugation but rather to the mechanical factor that the two sets of stylets are always applied to one another so firmly as to hold the tips of the stylets at the annulus, so that when one is absent the tip of the remaining one lacking the usual support cannot be readily brought to the middle line of the body. Moreover it is possible that the triangle will not be well applied to the orifice unless the fellow triangle be there to shove against it, as both are packed in side by side between the necks of the first stylets.

#### SUMMARY

Though the sperm of the crayfish, *Cambarus affinis*, is injured by exposure to water, it is transferred from the male to the female under water and stored up in an external pouch.

The part played by the female in this insurance against injury in transit has been elsewhere described.

The present paper describes only those organs of the male that are combined to form a safe conduit for the sperm from the male to the receptacle on the female.

The actual sperm transit apparatus of the male consists of three organs on each side of the body. The anatomy and use of these three organs are here described in detail.

The 'papilla' or end of the deferent duct is provided with glands and a valve. It is distended by blood and applied to fit accurately to the beginning of a tube.

This tube is the innermost part of the groove of the first stylet, or limb of the abdomen, and hitherto its existence and use has not been described.

The first abdominal limb is, in action, a duct leading the sperm uninterruptedly from the deferent duct into the receptacle of the female. It contains large glands of problematical use, and relies for mechanical support upon the habit of the male in using the second abdominal limb as well as one of the fifth thoracic limbs to insure the entrance of the first stylet into the receptacle of the female.

The second stylet is accessory to the first in applying its hand-like outgrowth over the papilla and insuring a tight joint. It also gives mechanical support to the first stylet. How much it may also serve as a piston for cleaning the tube or even for aiding in sperm transfer is left undecided.

The ontogeny of the first stylet shows that it begins after the other abdominal limbs and is from the first a simple unbranched outgrowth which becomes a tube by the depression of its central and elevation of its lateral parts to form a deep groove, the bottom of which is ultimately isolated by a shelf.

The morphology of the organ, based upon its use, anatomy and development, gives the basis for its utilization in defining species and subgenera. The tip or canula that is inserted into the receptacle to discharge sperm is the real tip of the organ and all other tips are to be referred to lateral outgrowths from one or the other side of the original groove.

The ontogeny of the second stylet shows that in the first larva it is just like the following abdominal limbs; but its subsequent

fate is to add on a lateral outgrowth (*appendix masculina*) which becomes the useful part of this organ when acting as a necessary part of the sperm transit apparatus.

The duplication of all three organs, right and left, seems necessary in as far as removal of one set leads to the lack of necessary mechanical support for the perfect functioning of the opposite set.

The evidence is against the conclusion that the right and left openings of different receptacles upon different females are necessarily met by the males employing the stylets of one side rather than an other. In each case the male may by trial obtain the entrance of some one of the two stylets into the receptacle of the female.

The extreme solidity of the shell of the stylets is to be correlated with the amount of force exerted by the male in making a water tight passage for the sperm from the deferent duct into the receptacle of the female.

While all six organs are necessary for sperm transfer, most of them may be removed without preventing the males from carrying out many of the stages of conjugation that would normally lead up to sperm transfer.

Many of the peculiarities of the form and structure of the transfer organs are demonstrated to be of use, or even necessary.

The accurate interadjustment of the six organs is necessary for the perpetuation of the species.

It is difficult to believe that in the evolution of *Cambarus* the increasing perfection of these organs could have been decisive in eliminating the less perfect organs. *Astacus* survives with more simple organs and the majority of genera of crayfish have no stylets at all. The perfection of the organs, characteristic of *Cambarus* may have been brought about from laws of change that it will require much experimentation to discover.

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## PLATES 1, 2, 3, 4

### EXPLANATION OF FIGURES

I. Photograph taken with a magnification of about ten diameters, of the posterior face of the first stylet of the left side.

II. Photograph of the same, taken from the median side, but diagonally, so that the posterior side is also shown in part.

III. Photograph of the same from the anterior face.

IV. Photograph of the same from the external face.

V. Photograph taken enlarged about ten diameters, of the second, or accessory stylet, of the left side of adult male. Posterior face.

VI. The same from the median face.

VII. The same from the anterior face.

VIII. The same from the external face.



I



II

E. A. ANDREWS



III



IV



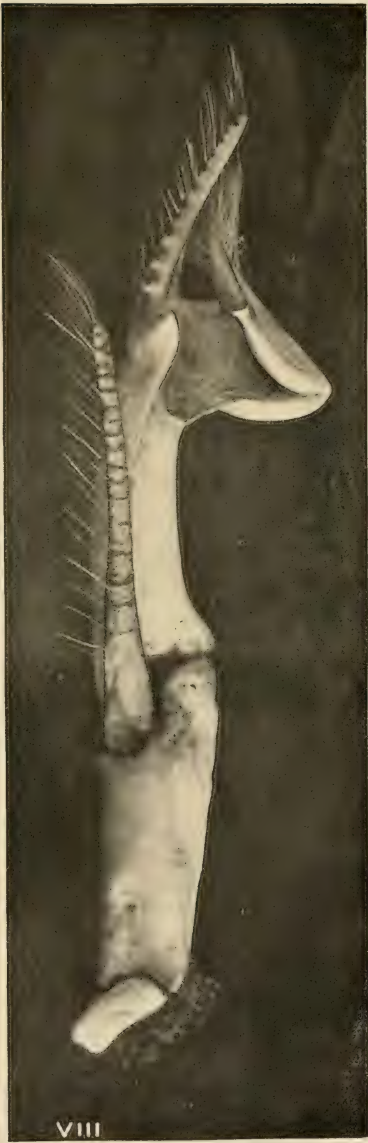
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VI



VII



VIII



## OVIPOSITION INDUCED BY THE MALE IN PIGEONS

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The influence of the male upon the time of oviposition is a matter in regard to which pigeons differ from some other birds, notably the domestic fowl. With regard to the fowl I have consulted a number of poultry keepers and experts, chiefly Dr. Raymond Pearl and Dr. Frank M. Surface, of the Maine Agricultural Experiment Station, where the most extensive studies of the egg-laying of fowls have been, and are being carried on. Dr. Pearl and Dr. Surface tell me that the domestic hen, and also the hen of the wild *Gallus bankiva* so far as can be ascertained, commence their spring laying at an approximately fixed date which can neither be deferred by withholding the cock nor advanced by giving the cock before the usual time.

Pigeons differ widely from poultry in this respect. If, from the winter season onward, an old female pigeon be kept unmated and isolated, she refrains from egg-laying, in evident distress for want of a mate, until the breeding season is far advanced; at length she does begin to lay, but her laying without a mate manifestly partakes of the abnormal. And a virgin pigeon, if kept isolated from other pigeons, may postpone her laying for a still longer period. On the other hand, a female pigeon, young or old, will lay very early in the season if she be early mated. Moreover, there is a pretty definite interval between the first copulation and the laying of the first egg, namely six or seven days; if the egg be delayed much beyond this time, the fact indicates some indisposition on the part of the female. And as the pair rear brood after brood throughout the season, this time-relation between copulation and egg-laying is regularly repeated.

The utility of this time adjustment in pigeons seems obvious. The male pigeon takes his turn daily in the duty of incubation: hence the female must not lay the eggs before he is ready to sit. This aspect of the matter, which has to do with pigeon sociology, has already been treated elsewhere (Craig '08) and will be discussed more fully in a book dealing with pigeon behavior. The present paper is to show, not why the male should determine the time of oviposition, but how he does determine it.

The thesis of the present paper is, that the influence of the male in inducing oviposition is a psychological influence; that the stimulus to oviposition is not the introduction of sperm, for the male can cause the female to lay even though he does not copulate with her. This is easily proven by an experiment, which requires only pigeons, patience, and time, and I shall now recount seven repetitions of such experiment, the first two being accidental cases, the other five being trials designed and carried out on purpose to test the thesis.

*Case 1 (1903).* In the spring of 1903 I brought together a virgin female dove (individual female no. 7, the species in all these trials being the blonde ring-dove, *Turtur risorius*) and a young inexperienced male, intending simply that they should mate in the normal manner. The young male played up to the female, but due to his inexperience and to other causes which need not be discussed here, his mating behavior was imperfect and he did not copulate with her. Nevertheless, in due time (six days) she laid an egg, and a second egg, as usual, forty hours later. This was the first intimation to me that a male bird can stimulate the female to lay, without copulating with her. Such an explanation seemed so absurd at that time that I dismissed it with the assumption that the birds must have copulated unobserved, and I did not even test the eggs to see if they were fertile. Looking back on that case now, however, and considering the observed behavior of that male, I feel reasonably certain that he did not fertilize the eggs but simply stimulated oviposition through the psychic (neural) channels.

*Case 2 (1904).* A female dove (no. 5) had been kept alone ever since her mate had died in November, 1903, and as time wore on

she showed intense anxiety to mate. She being a very tame bird, I had often caught and held her gently, but she did not like to be held, so one day in early March I tried tickling her head and pulling the feathers about her neck somewhat as a courting male would do it, and, finding that the poor lonely bird received these attentions with intense pleasure and became still more tame, I continued to preen her neck daily. She now acted toward the hand as if it were a mate, went through a nesting performance in her seed dish, there being no nest in her cage, and to my astonishment laid her eggs in due season. The first egg was laid March 11 and the second March 13. There is no doubt in my mind that the caressing of this bird's head and neck brought on oviposition. I once tried to repeat the experiment with another female dove, but she would not accept the touch of the hand as the former dove had done. Yet there is other evidence indicating that, with a specially tamed bird, this experiment, inducing oviposition by the hand, could be successfully repeated.

This case called to mind that of 1903, and suggested an experiment to determine definitely whether the male dove can stimulate the female to lay, without actual copulation. Opportunity to try this experiment was not found till 1907 and following years, when it was planned as follows.

#### *Method of the regular trials*

The experiment requires an unmated female dove that is not laying eggs, preferably a young dove that has never laid. It is best tried early in the season (*e.g.*, in February), especially if an old dove be used, for, as said above, if the female is kept too long without a mate she may lay without one. Side by side with this female, in a separate cage, is placed an unmated male, and the two are given several days to become acquainted. When they act toward one another like mate and mate, the doors separating them are opened and they are allowed to come together for a time, under constant supervision. When they attempt to copulate, a slender rod which can be thrust between the bars of the cage is used to keep them apart. Such attempts are made many times in a day,

mostly in the afternoon, and are continued for several days in succession; hence it is best that the experimenter should be able to devote some hours a day for several days in succession to a single pair or at most two pairs of birds. Whenever the birds are not under surveillance they are shut apart, each in his or her own cage. But they should be allowed to come together daily until the egg is laid.

A factor which caused difficulty in one of my trials was the nest. In cases 1, 2, 3 and 6, the bird laid without any nest at all (except that in case 6 a nest was given just a few hours before the egg was deposited). But in case 4 (*q.v.*) the female refused to lay without a nest: it was then necessary to remove the male and make the trial again, first giving the female a nest, and waiting long enough to prove that the nest alone would not cause her to lay.

#### *Results of the regular trials*

*Case 3* (1907). Female dove, no. 20. This bird had been bought recently from a dealer, and it was not known whether she had laid earlier in the season. But she was kept isolated for some time, during which she showed no inclination to lay. She was then given a male in the manner indicated. No nest given.

June 9. Male allowed in cage of female, and plays up to her.

June 15. First egg.

June 17. Second egg. (The second egg was of no special interest. After the first egg was laid, I generally left the doors open, allowing the pair to come together without surveillance.)

*Case 4* (1908). Female, the same. She had not laid since the close of last season. No nest given.

February 4. Male allowed to enter.

The female was unresponsive and showed by her behavior that this time she was holding back for want of a nest. This deficiency was supplied in the following manner (*vide ut supra.*)

February 8. Male taken away to another building.

March 10. Nest put in cage. Female paid practically no attention to it. Many days were allowed to pass, in order to make sure that the nest alone would not stimulate the female to lay.

March 21. Male (after short period in sight of female, that they might become re-acquainted) allowed to enter.

March 27. Egg laid.

*Case 5* (1910). Female, the same as in cases 3 and 4. She has laid no eggs since last season (1909.)

January 20. I begin to allow male in cage, at same time putting nest in.

January 29. Egg laid.

*Case 6* (1908). Female, no. 19. Virgin, has never laid. No nest given. In this case, the date on which the female was first given the requisite stimulus cannot be stated so definitely as in the other cases.

July 12. Male, in his cage, placed close to cage of female. Cooing commences. Female so excited that she several times assumes, and maintains in extreme degree, the copulation posture.

July 14. Male allowed into cage of female, but he fights her, so that it is necessary to remove him (otherwise the female might be painfully injured), and to allow the pair a few days more of preliminary acquaintanceship.

July 18. Male allowed to begin his series of daily visits.

July 22. Egg laid.

*Case 7* (1910). Female no. 19, the same as in case 6. She has laid no eggs since last summer (1909.)

For several days before contact with the male, a nest was kept in her cage; but she paid no attention to it, showing that the nest alone would not stimulate her to lay.

January 20. Male allowed to enter.

January 26. Egg laid.

#### SUMMARY

1. In six cases, stimulation of a female dove by a male, without copulation, was followed by oviposition; and in one other instance (case 2), stimulation by the hand of man in imitation of a male dove was followed by oviposition

2. In six of the seven cases (being all except case 3, in which the previous history was unknown), it was known that the female

had laid no eggs previously during the current year. In two of these six cases the dove was a virgin and had never laid.

3. It is true that the female may, if left without a mate, begin to lay late in the season. Hence it might be suspected that the sequence of stimulation and egg-laying in the seven cases was mere coincidence. But this is precluded, first of course by the fact that coincidences are not known to happen seven times in succession, and further by the following considerations.

4. In some of the trials it was proven that the female when stimulated by the male laid much earlier in the season than she did when not so stimulated. This is shown in the following table.

Female, no. 20.

1908. (Case 4), stimulated by male, laid March 27.

1909. (Control), without male, began to lay May 13.

1910. (Case 5), stimulated by male, laid January 29.

Female, no. 19.

1909. (Control), without male, began to lay April 26.

1910. (Case 7), stimulated by male, laid January 26.

5. The interval between the first stimulation by the male, and the laying of the first egg, was as follows:

Case 1. 6 days.

Case 2. (Male not used.)

Case 3. 7 days.

Case 4. 6 days.

Case 5. 9 days.

Case 6. 4 to 10 days, depending on what is regarded as the first stimulation in this case.

Case 7. 6 days.

The average and the variation of these intervals tally closely with the average and the variation of the interval in normal breeding, between the first copulation and the laying of the first egg.

6. There were no exceptions. Ovoposition never failed to follow within nine days after the first contact with the male. (The only partial failure was that of the first trial in case 4, which was due to faulty experimental conditions.)

## CONCLUSION

These facts make it certain that the male dove can stimulate the female to lay, without copulating with her.

Harper ('04) mentioned the fact that ovulation in the pigeon does not take place until after the bird is mated, but he was in doubt as to how far the influence of mating was a 'mental' one and how far it was a matter of the introduction of sperm. The present paper goes to show that the stimulus to the whole process of egg development and laying is a psychic (neural) stimulus, not dependent upon the introduction of sperm.

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## THE ANT-COLONY AS AN ORGANISM<sup>1</sup>

WILLIAM MORTON WHEELER

As a zoologist, reared among what are now rapidly coming to be regarded as antiquated ideals, I confess to a feeling of great diffidence in addressing an audience so thoroughly versed in the very latest as well as the very oldest biological facts, methods and hypotheses. I feel, indeed, like some village potter who is bringing to the market of the metropolis a pitiable sample of his craft, a pot of some old-fashioned design, possibly with a concealed crack which may prevent it from ringing true. Although in what I have to say, I shall strenuously endeavor to be modern, I can only beg you, if I fail to come within hailing distance of the advance guard of present day zoölogists, to remember that the range of adaptability in all organisms, even in zoölogists, is very limited.

Under the circumstances, my only hope lies in appealing to our permanent common biological interests and these, I take it, must always center in the organism. But the point of view from which we study this most extraordinary of nature's manifestations, is continually shifting. Twenty years ago we were captivated by the morphology of the organism, now its behavior occupies the foreground of our attention. Once we thought we were seriously studying biology when we were scrutinizing paraffine sections of animals and plants or dried specimens mounted on pins or pressed between layers of blotting paper; now we are sure that we were studying merely the exuviae of organisms, the effete residua of the life-process. If the neovitalistic school has done nothing else, it has jolted us out of this delusion which was gradually taking possession of our faculties. It is certain that whatever changes may overtake biology in the future, we must henceforth grapple

<sup>1</sup> A lecture prepared for delivery at the Marine Biological Laboratory, Woods Hole, Mass., August 2, 1910.

with the organism as a dynamic agency acting in a very complex and unstable environment. In using the term organism, therefore, I shall drop the adjective 'living,' since I do not regard pickled animals or dried plants as organisms.

As I wish to describe a peculiar type of organism, I may be asked, before proceeding, to state more concisely what I mean by an organism. It is obvious that no adequate definition can be given, because the organism is neither a thing nor a concept, but a continual flux or process, and hence forever changing and never completed. As good a formal definition as I can frame is the following: An organism is a complex, definitely coördinated and therefore individualized system of activities, which are primarily directed to obtaining and assimilating substances from an environment, to producing other similar systems, known as offspring, and to protecting the system itself and usually also its offspring from disturbances emanating from the environment. The three fundamental activities enumerated in this definition, namely nutrition, reproduction and protection seem to have their inception in what we know, from exclusively subjective experience, as feelings of hunger, affection and fear respectively.

Biologists long ago constructed an elaborate hierarchy of organisms. Those of a speculative turn of mind, like Spencer and Weismann, postulated the existence of very simple organisms, the physiological units, or biophores, which, though invisible, were nevertheless conceived as combining the fundamental activities above enumerated. These biophores were supposed to form by aggregation the cells, which may exist as independent organisms in the Protozoa and Protophyta or unite with other cells to form more complex aggregates, for which Haeckel's term 'persons' may be adopted. The person may be merely a cell-aggregate or consist of complexes of such aggregates as the metameres of the higher animals, for the separate metameres, according to a very generally accepted theory, are supposed to be more or less modified or highly specialized persons. Somewhat similar conditions are supposed to obtain in the composition of the vascular plants. The integration both of the metameric and non-metameric Metazoa may proceed still further, the simple persons combining to

form colonies in which the persons are primarily nutritive and acquire fixed and definite spatial relations to one another, whereas the more specialized animals, like the social insects, may constitute families of mobile persons with reproduction as the 'Leitmotiv' of their consociation. In man we have families associating to form still more complex aggregates, the true societies. Other comprehensive organisms are the cœnobioses, or more or less definite consociations of animals and plants of different species, which the ecologists are endeavoring to analyze. Finally we have philosophers, like Fechner, stepping in with the assertion, that the earth as a whole is merely a great organism, that the planetary systems in turn are colonies of earths and suns and that the universe itself is to be regarded as one stupendous organism. Thus starting with the biophore as the smallest and ending with the universe as the most comprehensive we have a sufficiently magnificent hierarchy of organisms to satisfy even the most zealous panpsychist. As biologists we may, for present purposes, lop off and discard the ends of this series of organisms, the biophores as being purely hypothetical and the cosmos as involving too many ultrabiological assumptions. We then have left the following series: first, the Protozoon or Protophyte, second the simple or non-metameric person, third the metameric person, fourth the colony of the nutritive type, fifth the family, or colony of the reproductive type, sixth the cœnobiote, and seventh the true, or human society. Closer inspection shows that these are sufficiently heterogeneous when compared with one another and with the personal organism, which is the prototype of the series, but I believe, nevertheless that all of them are real organisms and not merely conceptual constructions or analogies. One of them, the insect colony, has interested me exceedingly, and as I have repeatedly found its treatment as an organism to yield fruitful results in my studies, I have acquired the conviction that our biological theories must remain inadequate so long as we confine ourselves to the study of the cells and persons and leave the psychologists, sociologists and metaphysicians to deal with the more complex organisms. Indeed our failure to coöperate with these investigators in the study of animal and plant societies has blinded us to many aspects of the

cellular and personal activities with which we are constantly dealing. This failure, moreover, is largely responsible for our fear of the psychological and the metaphysical, a fear which becomes the more ludicrous from the fact that even our so-called 'exact' sciences smell to heaven with the rankest kind of materialistic metaphysics.

Leaving these generalities for the present, permit me to present the evidence for the contention that the animal colony is a true organism and not merely the analogue of the person. To make this evidence as concrete as possible I shall take the ant-colony as a paradigm and ask you to accept my statement that the colonies of the termites, social bees and wasps, which the limited time at my disposal does not permit to consider, will be found to offer the same and in some cases even more satisfactory data. I select the ant-colony not only because I am more familiar with its activities, but because it is much more interesting than that of the polyps, more typical and less specialized than that of the honey bee, less generalized than that of the wasps and bumble-bees, and has been much more thoroughly investigated than the colonies of the stingless bees and the termites.

The most general organismal character of the ant-colony is its individuality. Like the cell or the person, it behaves as a unitary whole, maintaining its identity in space, resisting dissolution and, as a general rule, any fusion with other colonies of the same or alien species. This resistance is very strongly manifested in the fierce defensive and offensive coöperation of the colonial personnel. Moreover, every ant-colony has its own peculiar idiosyncrasies of composition and behavior. This is most clearly seen in the character of the nest, which bears about the same relation to the colony that the shell bears to the individual Foraminifer or mollusc. The nest is a unitary structure, built on a definite but plastic design and through the coöperation of a number of persons. It not only reflects the idiosyncrasies of these persons individually and as a whole, but it often has a most interesting adaptive growth and orientation which may be regarded as a kind of tropism. In many species the nest mounds, which are used as incubators of the brood and as sun-parlors for the adult ants, are constructed in

such a manner as to utilize the solar radiation to the utmost. In the Alps and Rocky Mountains we find the nests oriented in such a manner that the portions in which the brood is reared face south or east, and as time goes on the nests often grow slowly in these directions, like plants turning to the light, so that they become greatly elongated. This orientation is, in fact, so constant in some species that the Swiss mountaineers, when lost in a fog, can use it as a compass.

Every complete ant-colony, moreover, has a definite stature which depends, of course, on the number of its component persons. And this stature, like that of personal organisms, varies greatly with the species and is not determined exclusively by the amount of food, but also by the queen mother's fertility, which is constitutional. Certain ants live in affluence but are nevertheless unable to form colonies of more than fifty or a hundred individuals, while others, under the same conditions, have a personnel of thousands or tens of thousands.

One of the most general structural peculiarities of the person is the duality of its composition as expressed in the germ-plasm on the one hand and the soma on the other, and the same is true of the ant-colony, in which the mother queen and the virgin males and females represent the germ-plasm, or, more accurately speaking, the 'Keimbahn,' while the normally sterile females, or workers and soldiers, in all their developmental stages, represent the soma. In discussing the question of the inheritance or non-inheritance of acquired characters the Neodarwinians trace all the congenital modifications of the worker and soldier phases to the queen, just as in the personal organism all the congenital somatic characters are traced to the germ-plasm of the egg. Since the homologue of the reproductive organ of the ant-colony consists of the virgin males and females, and since the males mature earlier than the females, the colony may be regarded as a protandric hermaphrodite. Some colonies, however—and this is probably characteristic of certain species—produce only males or females and are therefore in a sense gonochoristic, or dioecious. And this protandric hermaphroditism and gonochorism, like the corresponding conditions in persons, may be interpreted as a device for, or, at

any rate, as an aid, in insuring cross-fertilization. The fecundated queen of the ant-colony represents the first link in the 'Keimbahn' and therefore corresponds to the fertilized egg of the personal organism. She produces both the worker personnel and the virgin males and females, just as the fertilized egg produces both the soma and the germ-cells. The colonial soma, moreover, may be differentiated as the result of a physiological division of labor into two distinct castes, comprising the workers in which the nutritive and nidificational activities predominate, and the soldiers, which are primarily protective. Here, too, the resemblance to the differentiation of the personal soma into entodermal and ectodermal tissues can hardly be overlooked.

The structure of the ant-colony thus appears to be very simple as compared with that of its component persons. The question naturally arises as to the particular type of unicellular or personal organism which it most resembles. Undoubtedly, if we could see it acting in its entirety, the ant-colony would resemble a gigantic foraminiferous Rhizopod, in which the nest would represent the shell, the queen the nucleus, the mass of ants the plasmodium and the files of workers, which are continually going in and out of the nest, the pseudopodia.

The ant-colony, of course, like the person, has both an ontogenetic and a phylogenetic development; the former open to observation, the latter inferred from the ontogeny, a comparison of the various species of ants with one another and with allied Hymenopterous insects, and from the paleontological record. The fecundated queen, as I have stated, represents the fertilized egg which produces the colonial organism, but she is a winged and possibly conscious egg, capable not only of actively disseminating the species, like the minute eggs of many marine animals, but of selecting the site for the future colony. After finding this site she discards her wings and henceforth becomes sedentary like the wingless workers which she will produce. The whole colony rests satisfied with the nesting site selected by its queen if the environmental conditions remain relatively constant. If these become unfavorable, however, the colony will move as a whole to a new site. In most species such movements are rather limited, but the

nomadic driver and legionary ants are almost continually moving from place to place and must cover a considerable territory during the year. After the queen has selected the nesting site, she immures herself in some earthen or vegetable cavity, lays a number of eggs, supplying them with yolk derived by metabolism from her fat-body and now useless wing-muscles, and feeds the hatching larvæ on her salivary secretion, which, though highly nutritious, is, nevertheless, very limited in quantity, so that the offspring when mature are dwarfed and very few in number. They are in fact, workers of the smallest and feeblest caste; but they set to work enlarging the nest, break through the soil or plant tissues, construct an entrance on the surface and seek food for themselves and their famished mother. This food enables her to replenish her fat-body and to produce more eggs. Her expansive instincts and activities now contract, so to speak, and become reduced henceforth to a perpetual routine of assimilation, metabolism and oviposition. She produces brood after brood during her long life which may extend over a period of ten to thirteen years. Her workers assume the duties of foraging, of feeding the larvæ and one another, and of completing the nest. Their size and polymorphism increase with successive broods, till the soldier forms, if these are characteristic of the species, make their appearance. Then the individuals which correspond to the reproductive cells of the personal organism, namely, the virgin males and females develop, and the colonial organism may be said to have reached maturity. Like the personal organism, it may persist for thirty or forty years or, perhaps, even longer without much growth of its soma, since the workers and soldiers of which this consists are exposed to many vicissitudes and live only from three to four years and probably, as a rule, for a much shorter period. If the queen grow too old or die the colony, as a rule, dwindles and eventually perishes unless her place is taken by one or more of her fertile daughters.

This is the ontogenetic history of most ant-colonies. It is so similar to the phylogenetic history derived from the sources mentioned above that we have no hesitation in affirming that it conforms in the most striking manner to the biogenetic law. The

very ancient behavior of the solitary female Hymenopteron is still reproduced during the incipient stage of colony formation, just as the unicellular phase of the Metazoon is represented by the egg. A further correspondence of the ontogeny and phylogeny is indicated by the fact that the most archaic and primitive of living ants form small colonies of monomorphic workers closely resembling the queen, whereas the more recent and most highly specialized ants produce large colonies of workers not only very unlike the queen but unlike one another.

In order to complete the foregoing account it will be necessary to consider some interesting modifications of the usual method of colony formation and growth, especially as these modifications furnish additional and striking evidence in favor of the contention that the ant-colony is a true organism. In many species, after the colony has reached maturity and especially if the food-supply continue to be abundant, several of the virgin females may be fecundated in the nest, lose their wings and remain as members of the colony. This may, indeed, contain half a dozen and in extreme cases as many as forty or fifty or even more fertile queens. But often the growth of the colonial organism becomes excessive through an increase in the worker personnel and passes over into a form of colonial reproduction, when the young fertilized queens, each accompanied by a band of workers, start new nests in the vicinity of the parental formicary. In this manner a very large and complex colony may arise and extend over many adjacent nests. For some time the new settlements may remain in communication with the home-nest through files of workers, but eventually the daughter settlements may become detached and form independent colonies. The resemblance of this method of reproduction, which is essentially the same as the swarming in the honey-bee, to the asexual reproduction of many unicellular and multicellular organisms by a process of budding, is too obvious to need further comment.

The important rôle of nutrition in the development of the colony will be clear from the foregoing remarks. It becomes even more striking in the methods adopted by the queens of certain parasitic species in starting their colonies. Some European

observers and myself have found a number of queen-ants that are unable to found colonies without the aid of workers of allied species. These queens may be separated into four groups, as follows:

1. The queen which enters a colony of an alien species and decapitates its queen or is the occasion of her being killed off by her own workers. The intrusive queen is then adopted by the workers and a compound colonial organism arises, consisting of the germ-plasm of one species and the soma of another. The queen proceeds to lay eggs, which are reared by the alien workers, thus relieving her of all the labor and exhaustion endured by the independent typical ant-queen during the early stages of colony formation. *Pari passu* with the development of the worker offspring of the intrusive queen, the worker nurses grow old and die, so that the colony eventually comes to consist of only one species, the soma of the host being replaced by that of the parasite. This method of colony formation, first observed among our American ants and later among certain European and North African species, I have called temporary social parasitism. Now many of the species, which behave in this manner, have extremely small queens, or queens provided with a peculiar pilosity or sculpture that tend to endear them to the workers of the alien colonies which they invade. If we regard the large fertilized queens of ordinary ants, which are supplied with a voluminous fat-body and wing-musculature, as representing eggs provided with a great amount of yolk, and the diminutive queens of the temporary social parasites as the equivalents of alecithal eggs, we have another striking resemblance between the personal and colonial organisms, for the large queens, like the yolk-laden eggs of many vertebrates, are produced in small numbers but are able to generate the colonial soma independently, whereas the small queens, which are produced in great numbers, in order that some of them may survive the vicissitudes of a parasitic life, correspond to the small yolk-less eggs of many parasites, which have to be deposited in plant or animal tissues in order that the imperfect young on hatching may be surrounded by an abundance of food.

2. The queen of the blood-red slave-maker (*Formica sanguinea*) adopts a different method. She enters the colony of an

allied species, snatches up the worker brood and kills any of the workers or queens that endeavor to dispute her possessions. The ants hatch with a sense of affiliation with their foster mother and proceed to rear her eggs and larvæ as soon as they appear. Here, too, the colony is formed by a mixture of two species, but the workers produced by the intrusive queen inherit her predatory instincts and therefore become slave-makers. They keep on kidnapping worker larvæ and pupæ from the nests of the alien species, carry them home, and eat some of them but permit many to mature, so that the mixed character of the colony is maintained. This, however, is not invariably the case, for old and vigorous sanguinea colonies may cease to make slave-raids and the slaves may die off and leave a pure colony of the predatory species. The advantages of this method of colony formation are obvious, for the colonial soma, being composed of two species, grows more rapidly and is much more efficient as a nutritive and protective support to the colonial germ-plasm, which is restricted to the predatory species.

3. The colony-founding queen of the amazon ants of the genus *Polyergus* resorts to a modification of the method adopted by *sanguinea*, as has been shown by Emery's recent observations. She enters the colony of an alien species, perforates its queen's head with her sickle-shaped mandibles and permits herself to be adopted by the workers. She pays no attention to the brood but begins to lay eggs, the larvæ from which are carefully reared by the workers. The *Polyergus* offspring inherit the pugnacity of their mother, but, like the *sanguinea* workers, have the ability to kidnap the brood of other ants. They are, in fact, slave-makers of a very deft and ferocious type. Like their mother, however, they are unable to excavate the nest, to care for their own young or to take food except from the mouths of the workers that hatch from the kidnapped larvæ and pupæ. The mixture of the two species is therefore obligatory, and the slave personnel, which represents the nutritive and nest-building portions of the colonial soma, has to be maintained throughout the life of the colony.

4. Certain feeble queen ants belonging to a few aberrant genera (*Anergates*, *Wheeleriella*) invade populous nests of an alien species and are adopted in the place of their queens, which are

destroyed by their own workers. The parasites then proceed to lay eggs but these give rise only to males and females as the worker caste is entirely suppressed. The colony retains a mixed character, the parasitic species usurping the functions of the germ-plasm, while the host is purely somatic. As there are no means of prolonging the lives of the host-workers and as they do not reproduce, the whole colony is short-lived and the maturation of the parasitic sexual individuals has to be accelerated so that it will fall within the brief life-time of the worker hosts. This condition I have called permanent social parasitism.

These four peculiar types of colony-formation all lead to the formation of compound colonial organisms, comparable to certain compound personal organisms which, with few exceptions, can be produced only by artificial means. In temporary social parasitism the colonial egg can develop its soma only when grafted on to the soma of another species. This soma eventually perishes and the colony then assumes a normal complexion. This condition reminds us of certain tropical plants, like the species of *Clusia* and *Ficus*, which develop as epiphytes on other trees but after killing their hosts take root in the soil and thenceforth grow as independent organisms. The slave-makers of the sanguinea or facultative type are also unable to develop the soma except when grafted on to the soma of another species, but in this case the co-operation of both somas in nourishing and protecting the germ-plasm is maintained for a much longer period. This kind of colony may be compared with a graft made by uniting the longitudinal half of one plant with that of another so that both take nourishment through their roots. To make the resemblance more complete one of the grafted halves would have to be pruned in such a manner as to prevent flowering. In the amazons or obligatory slave-makers and the permanent social parasites the alien soma alone has a nutritive function, so that the conditions are like those in ordinary vegetable grafts, in which the stock retains the roots and the scion produces the flowers and fruit.

I have dwelt on the various methods of colony formation not only because they give us an insight into colonial reproduction, but because they throw light on the colonial organism from the

standpoint of parasitology. That the four types of queens and their offspring are directly comparable with entoparasitic persons is not so remarkable as the fact that in ants the host and parasite form a mixed organism which could only be obtained with persons by jumbling together the component cells of host and parasite like two kinds of peas shaken in a bottle. Notwithstanding this mixture the parasitic colony not only retains its identity and the anticipatory character of its behavior but castrates the host colony and constrains its soma either to coöperate in many of its activities or to specialize as a purely nutritive or nest-building auxiliary. The host is thus reduced to the status of a nourishing or protective organ of the parasite. This behavior has many striking analogies among persons. Giard long ago called attention to the fact that when the cirriped *Sacculina* settles under the abdomen of a male crab and sends its rootlike haustoria into the tissues of its host, the latter undergoes castration, and its narrow abdomen expands to form a protection for the soft-bodied parasite. In other words, the parasite acts as if it were a mass of crabs' eggs and the male crab behaves as if it had changed its sex and develops an abdomen of the female type.

Not only are there ants, like those already considered, that may be regarded as colonial entoparasites, but there are also a number of species that may be called colonial ectoparasites. These form the so-called 'compound nests,' in which two or more species live amicably side by side, or may even mingle freely with one another, but rear their broods in separate nests, thus indicating in the clearest manner the integrity of the colonial organism. This is also shown by the vast number of myrmecophilous insects, which are, of course, ento- or ectoparasitic persons, and behave towards the ant colony as if it were a rather incoherent and therefore more vulnerable, or exploitable personal organism.

Finally we come to what the neovitalists regard as the most striking autonomic manifestations of the organism, namely the regulations and restitutions, and face the question as to whether these, too, have their counterpart in the colonial organism. I believe that the following facts compel us to answer this ques-

tion in the affirmative. If the worker personnel be removed from a young ant-colony, leaving only the fertile queen, we find that this insect, if provided with a sufficiently voluminous fat-body, will set to work and rear another brood, or, in other words, regenerate the missing soma. And, of course, any portion of the worker or sexual personnel, that is removed from a vigorous colony will be readily replaced by development of a corresponding portion of the brood. On the other hand, if the queen alone be removed, one of the workers will often develop its ovaries and take on the egg-laying function of the queen. In ants such substitution queens, or gynaeceoid workers are not fertilized and are therefore unable to assume their mother's worker- and queen-producing functions. The termites, however, show a remarkable provision for restituting both of the fertile parents of the colony from the so-called complemental males and females. In ants we have a production of fertile from normally infertile individuals, but the incompleteness of the result does not disprove the existence of a pronounced restitutorial tendency.

Very striking examples of this tendency are exhibited when colonies are injured by parasitic myrmecophiles. I shall consider only the case of the peculiar beetle *Lomechusa strumosa*, which breeds in colonies of the blood-red slave-maker (*Formica sanguinea*). Though the beetle and its larvæ are treated with great affection, the latter devour the ant larvæ in great numbers, so that little of the brood survives during the early summer months when the colony is producing its greatest annual increment to the worker personnel. The ants seem to perceive this defect and endeavor to remedy it by converting all the surviving queen larvæ into workers. But as these larvæ have passed the stage in their development when such an operation can be successful, the result is the production of a lot of pseudogynes, or abortive creatures structurally intermediate between the workers and queens and therefore useless in either capacity. It is instructive to compare this case with the regeneration of the lens from the iris in the Amphibian eye. In his recent analysis of the stimuli of restitution in personal organisms Driesch reaches the conclusion that "the specificity of what is taken away certainly forms part of the

stimulus we are searching for, and it does so by being communicated in some way by something that has relations to *many*, if not *all*, parts of the organism and not only to the neighboring ones." He also says that "each part of the organism assigns its specific share to an unknown something and that this something is altered as soon as a part is *removed* or absolutely *stopped* in its functional life, and that the specific alteration of the something is our stimulus of restitutions." These quotations and Driesch's further discussion of the problem are even clearer in their application to the colonial than to the personal organism, for in the former it is much easier to see how each individual insect "can do more than one thing in the service of restitution" than it is to understand how each cell of the person can do more than one thing in restoring a lost organ.

I fear that I may have wearied you with this long attempt to prove that the ant-colony is a true organism, especially as this statement must seem to some of you to be too trite for discussion, but when an author like Driesch writes a large work in two volumes on the "Philosophy of the Organism" and ignores the colonial organisms altogether, an old-fashioned zoologist may perhaps be pardoned for calling attention to a well-founded, though somewhat thread-bare, biological conception.

If it be granted that the ant-colony and those of the other social insects are organisms, we are still confronted with the formidable question as to what regulates the anticipatory coöperation, or synergy of the colonial personnel and determines its unitary and individualized course. The resemblance of the ant- or bee-colony to the human state long ago suggested a naive reply to this question. Aristotle naturally supposed the colonial activities to be directed and regulated by a βασιλεύς or ἡγεμών, because these personages managed affairs in the Greek states. After the sex of the fertile individual had been discovered by Swammerdam, the word 'queen' was naturally substituted for βασιλεύς or 'king,' and as queens in human states do not necessarily govern and are often rather anabolic, sedentary and prolific persons and the objects of much flattering attention, the term is not altogether inapt when applied to the fertile females of insect colonies. It

has been retained although everybody knows that these colonies represent a form of society very different from our own, a kind of communistic anarchy, in which there is "neither guide, overseer nor ruler," as Solomon correctly observed. In this respect too, the colony is essentially the same as the personal organism, at least in the opinion of those who do not feel compelled to assume the existence of a 'soul' in the scholastic sense. For it is clear, that to primitive thinkers the soul was supposed to bear the same relation to the person as the *βασιλεύς* to the insect colony and the king to the human state. This supposition is still held though in a more subtle form, by writers of the present day. Some of these, like Maeterlinck, clothe the postulated controlling agency in a mystical or poetic garb and call it the 'spirit of the hive.' The following passage from the Belgian poet's charming account of the honey-bee will serve to illustrate this method of meeting the problem:

What is this 'spirit of the hive'—where does it reside? It is not like the special instinct that teaches the bird to construct its well planned nest, and then seek other skies when the day for migration returns. Nor is it a kind of mechanical habit of the race, or blind craving for life, that will fling the bees upon any wild hazard the moment an unforeseen event shall derange the accustomed order of phenomena. On the contrary, be the event never so masterful, the 'spirit of the hive' still will follow it, step by step, like an alert and quickwitted slave, who is able to derive advantage even from his master's most dangerous orders.

It disposes pitilessly of the wealth and the happiness, the liberty and life, of all this winged people; and yet with discretion, as though governed itself by some great duty. It regulates day by day the number of births, and contrives that these shall strictly accord with the number of flowers that brighten the country-side. It decrees the queen's deposition or warns her that she must depart; it compels her to bring her own rivals into the world, and rears them royally, protecting them from their mother's political hatred. So, too, in accordance with the generosity of the flowers, the age of the spring, and the probable dangers of the nuptial flight will it permit or forbid the first-born of the virgin princesses to slay in their cradles her younger sisters, who are singing the song of the queens. At other times, when the season wanes, and flowery hours grow shorter, it will command the workers themselves to slaughter the whole imperial

brood, that the era of revolutions may close, and work become the sole object of all. The 'spirit of the hive' is prudent and thrifty, but by no means parsimonious. And thus, aware, it would seem, that nature's laws are somewhat wild and extravagant in all that pertains to love, it tolerates, during summer days of abundance, the embarrassing presence in the hive of three or four hundred males, from whose ranks the queen about to be born shall select her lover; three or four hundred foolish, clumsy, useless, noisy creatures, who are pretentious, gluttonous, dirty, coarse, totally and scandalously idle, insatiable, and enormous.

But after the queen's impregnation, when flowers begin to close sooner and open later, the spirit one morning will coldly decree the simultaneous and general massacre of every male. It regulates the workers' labours with due regard to their age; it allots their task to the nurses who tend the nymphs and the larvæ, the ladies of honour who wait on the queen and never allow her out of their sight; the house-bees who air, refresh, or heat the hive by fanning their wings, and hasten the evaporation of the honey that may be too highly charged with water; the architects, masons, wax-workers, and sculptors who form the chain and construct the combs; the foragers who sally forth to the flowers in search of the nectar that turns into honey, of the pollen that feeds the nymphs and the larvæ, the propolis that welds and strengthens the buildings of the city, or the water and salt required by the youth of the nation. Its orders have gone to the chemists who ensure the preservation of the honey by letting a drop of formic acid fall in from the end of their sting; to the capsule makers who seal down the cells when the treasure is ripe, to the sweepers who maintain public places and streets most irreproachably clean, to the bearers whose duty it is to remove the corpses; and to the amazons of the guard who keep watch on the threshold by night and by day, question comers and goers, recognize the novices who return from their very first flight, scare away vagabonds, marauders and loiterers, expel all intruders, attack redoubtable foes in a body, and, if need be, barricade the entrance.

Finally, it is the spirit of the hive that fixes the hour of the great annual sacrifice to the genius of the race: the hour, that is, of the swarm; when we find a whole people, who have attained the topmost pinnacle of prosperity and power, suddenly abandoning to the generation to come their wealth and their palaces, their homes and the fruits of their labour; themselves content to encounter the hardships and perils of a new and distant country. This act, be it conscious or not, undoubtedly passes the limits of human morality. Its result will sometimes be ruin, but poverty

always; and the thrice-happy city is scattered abroad in obedience to a law superior to its own happiness. Where has this law been decreed which, as we soon shall find, is by no means as blind and inevitable as one might believe? Where, in what assembly, what council, what intellectual and moral sphere, does this spirit reside to whom all must submit, itself being vassal to an heroic duty, to an intelligence whose eyes are persistently fixed on the future?

It comes to pass with the bees as with most of the things in this world; we remark some few of their habits; we say they do this, they work in such and such fashion, their queens are born thus, their workers are virgin, they swarm at a certain time. And then we imagine we know them, and ask nothing more. We watch them hasten from flower to flower, we see the constant agitation within the hive; their life seems very simple to us, and bounded, like every life, by the instinctive cares of reproduction and nourishment. But let the eye draw near, and endeavour to see; and at once the least phenomenon of all becomes overpoweringly complex; we are confronted by the enigma of intellect, of destiny, will, aim, means, causes; the incomprehensible organization of the most insignificant act of life.

Other authors like Driesch, give the postulated controlling agency the sharper outlines of a would-be scientific but in reality metaphysical entity and call it the 'entelechy.' It is true that the entelechy is deduced by Driesch from the autonomic peculiarities of the personal organism, but as the colony has all the essential attributes of the organism, he would undoubtedly assign it an entelechy, which according to the definition would have to be nonspacial, but working into space, nonspychic, but conceivable only after analogy with the psychic, and non-energetic, but nevertheless capable of determining the specificity of the colonial activities through releasing and distributing energy.

I confess that I find the entelechy quite as useless an aid in unravelling the complex activities of the ant-colony as others have found it in analyzing the personal organism. This angel-child, entelechy, comes, to be sure, of most distinguished antecedents, having been mothered by the Platonic idea, fathered by the Kantian Ding-an-sich, suckled at the breast of the scholastic *forma substantialis* and christened, from a strong family likeness, after old Aristotle's darling *εντελέχεια*, but nevertheless, I believe that

we ought not to let it play about in our laboratories, not because it would occupy any space or interfere with our apparatus, but because it might distract us from the serious work in hand. I am quite willing to see it spanked and sent back to the metaphysical house-hold.

But, speaking seriously, it seems to me that if the organism be inexplicable on purely biological grounds, we should do better to resort to psychological agencies like consciousness and the will. These have at least the value which attaches to the most immediate experience. And even the subconscious and the super-conscious are more serviceable as explanations than such anaemic metaphysical abstractions as the entelechy. Of course, psychic vitalism is one of Driesch's pet aversions and he will have none of it, because he is a solipsist, but the fact that he is compelled to operate with a 'psychoid' and with an entelechy conceivable only *per analogiam* with the psychic, shows the inconsistency of his position.

Before we can adopt any ultrabiological agencies, however, except in a tentative and provisional manner, an old and very knotty problem will have to be more thoroughly elucidated. I refer to the problem of the correlation and coöperation of parts. If the cell is a colony of lower physiological units, or biophores, as some cytologists believe, we must face the fact that all organisms are colonial or social and that one of the fundamental tendencies of life is sociogenic. Every organism manifests a strong predelection for seeking out other organisms and either assimilating them or coöperating with them to form a more comprehensive and efficient individual. Whether, with the mechanists, we attribute this tendency to chemotropism or cytotropism, or with the psychic neovitalists, interpret it as conscious and voluntary, we certainly cannot afford to ignore the facts. The study of the ontogeny of the person, *i.e.*, the person in the process of making, in the hands of recent experimentalists, has thrown a flood of light on the peculiarities of organization, but the animal and plant colony are in certain respects more accessible to observation and experiment, because the component individuals bear such loose spacial relations to one another. Then too, the much simpler and more primi-

tive organismal type of the colony, as compared with that of the person, should enable us to follow the process of consociation and the resulting physiological division of labor more successfully. In the problem, as thus conceived, we must include, not only the true colony and society, and the innumerable cases of symbiosis, parasitism and cœnobiosis, but also the consociation and mutual modification of hereditary tendencies in parthenogenetic and biparental plants and animals, since in all of these phenomena our attention is arrested not so much by the struggle for existence, which used to be painted in such lurid colors, as by the ability of the organism to temporize and compromise with other organisms, to inhibit certain activities of the aequipotential unit in the interests of the unit itself and of other organisms; in a word, to secure survival through a kind of egoistic altruism.<sup>2</sup>

<sup>2</sup>Since this paragraph was written I have found that several recent authors have given more explicit expression to a very similar conception to the rôle of coöperation and struggle in the development of organisms. Especially worthy of mention in this connection are Kammerer (*Allgemeine Symbiose und Kampf ums Dasein als gleichberechtigte Triebkräfte der Evolution*. Arch. f. Rass. u. Ges.-Biol. 6, 1909, pp. 585-608), Schiefferdecker (*Symbiose*. Sitzb. niederrhein. Ges. f. Natur. u. Heilk. zu Bonn, 13, Juni, 1904, 11 pp.), Bölsche (*Daseinskampf und gegenseitige Hilfe in der Entwicklung*. Kosmos, 6, 1909); and Kropotkin (*Mutual aid, a factor of evolution*, London, 1902).



# SEXUAL ACTIVITIES OF THE SQUID, *LOLIGO* *PEALII* (LES.)

## I. COPULATION, EGG-LAYING AND FERTILIZATION

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THIRTEEN FIGURES

FOUR PLATES

This account, which deals with some of the sexual activities of the squid, is based upon observation made on specimens kept in glass sided aquaria at the Marine Biological Laboratory, Woods Hole, Mass. Specimens caught in the fish traps of the immediate vicinity may, by careful handling, be kept in aquaria in fairly good condition for a number of days. Such specimens occasionally copulate and eggs are sometimes laid.

There are two methods of copulation. By one method the spermatophores ejaculate their contents so the sperm reservoirs thrown from them are attached in a special depression on the inner side of the outer buccal membrane opposite the junction of the two ventral arms (figs. 8 and 10). They then slowly emit sperm, which are carried to and stored in, a special sperm receptacle that opens near this depression and is imbedded in the tissue of the outer buccal membrane (figs. 10 and 11). In this receptacle the sperm are mixed with a secretion and are not active. How long the sperm may be retained in the receptacle is not known, but there is some reason to think that they may be retained for at least some weeks. Females with eggs that can be fertilized may be found during the four months, June to late September, that I have worked at Woods Hole. Without exception every adult female that had not spawned had the sperm receptacle filled more or less completely with sperm, although in many cases the

eggs were far from mature. This, together with the dormant condition of the sperm in the receptacle, and the fact that they seem to be poured out only during egg laying, point to a possible long retention. It is certain that the same female may have sperm reservoirs attached near this receptacle a number of times after it has been filled, and it is possible that the same sperm do not continue long in the receptacle. There seems, however, to be no evidence that they are discharged except during the period of egg laying.

The other method of copulation results in fastening the sperm reservoirs of the ejaculated spermatophores near the end of the oviduct (fig. 8, s) usually directly on its walls but sometimes on the mantle, gill or visceral mass. There is no special receptacle for the sperm from these sperm reservoirs. They escape into the water, becoming active as they escape, and pass out with the water through the funnel. The escape of the sperm is rather rapid but there are vast numbers in each reservoir, from which they are constantly poured like smoke from a chimney until the reservoir is empty. It is not known how long it takes to empty a reservoir but by keeping reservoirs from spermatophores that ejaculated in dishes of sea-water, and by examining reservoirs normally attached to the oviducts and buccal membranes of females, it seems probable that the sperm do not all escape for two or more days.

In aquaria I have seen rather more cases of copulation where the spermatophores are inserted into the mantle chamber than where the sperm reservoirs are attached to the buccal membrane. This may be because of the limited quarters in aquaria. In the larger floating tanks, in which specimens are sometimes kept before they are brought into the laboratory, the buccal membrane copulation seems proportionally more common than in aquaria, but even here the mantle chamber copulation seems to be rather more frequent.

The same individuals may copulate several times in the course of a few hours. In general the male is aggressive. The female may attempt to escape or she may be quite passive. Spermatophores seem to be inserted in the mantle chambers of only those

females that are nearly ready to deposit their eggs. In the large number of trials made it was found that the eggs of these individuals were so nearly mature they could be artificially fertilized. Females that are nearly ready to deposit eggs have the nidamental glands considerably swollen and the accessory nidamental glands are highly colored with bright red. Wherever the spermatophores were inserted in the mantle chamber these glands were in this condition.

Before copulation both female and male are usually especially active and may be known as sexually excited animals by their peculiar movements. The female in swimming seems to be nervous or excited. She throws short but rapid puffs of water from the funnel, moves the tail fin very rapidly and, leaving the arms quite limp, spreads them apart and frequently throws them to one side. This gives the arms a jerky or trembling motion not shown in ordinary swimming. Except during the most rapid movements of the female, the male solemnly swims by her side, an inch or two away, but parallel, and with his head in the same direction. He frequently manipulates his arms, spreading them apart, commonly with the two dorsal arms elevated nearly or quite to a perpendicular position, and the third arms spread far to the sides (fig. 3). This position is not infrequently accompanied by localized activity of chromatophores. A spot may appear near the base of each third arm and a smaller spot on each second arm a little further from its base. These spots do not remain continuously while the male is in this attitude but suddenly appear with each increase of activity on the part of either the male or female. Occasionally blushing is quite general over the head and anterior end of the body and sometimes includes the whole body but the bodies of both animals generally remain colorless except for the special spots mentioned on the male. The attitude of the male, with elevated and spread arms, is not continuous but is assumed every few minutes, or in some cases seconds, and the arms may be brought into the usual position of a swimming animal for periods of many minutes.

Males do not all respond equally to the presence of sexually active females. Not uncommonly one male in an aquarium containing

several males will follow the females around by the hour while the other males remain entirely inattentive. Usually when a male begins to show sexual activity he will follow a single female although other females that show similar activities are present in the aquarium. Occasionally he may change to another individual but he nearly always returns after a few minutes to the one to which he has been paying chief attention.

A few males have been observed that were so sexually excited they followed individuals around quite indiscriminately. Under such conditions I have upon three occasions seen a male catch another male and insert spermatophores into his mantle chamber. Two of the three instances were between the same individuals, the second performance being only a few minutes after the first. In each of these cases the male seized made great efforts to get away and finally to get hold of the male that was holding him but was unsuccessful. Upon killing the male that received the spermatophores, sperm reservoirs were found attached to the base of the left gill and to the adjacent visceral mass. Such exceptionally active males may copulate repeatedly with a single female. In a few cases this has been carried so far that the female has actually been killed. Even after the female has become entirely inactive and apparently dead the male may copulate with her several times. In one case, a male that had been several days without food, after copulating with a weakened female, retained his hold and killed her by eating a considerable hole through the mantle.

The male always uses the same arm for transferring the spermatophores. This arm, the left ventral, is not greatly modified, but a short distance from its tip some of the suckers, especially those in the row farthest from the midline of the body, and a ridge between the rows of suckers show modification (fig. 4, *h*). The peduncles of a dozen or more of the suckers of the outer row are considerably elongated and the sucking discs of a few, (six or eight) are greatly reduced in size or entirely absent. In both directions from these, the discs become increasingly normal until no modification is apparent. The suckers of the row toward the midline of the body are somewhat modified, the peduncles being somewhat shorter than those of the other suckers in the row, and the suck-

ing discs somewhat smaller, but in none of the suckers of this row are the sucking discs entirely absent. A glandular plaited ridge extends lengthwise between the suckers of this region and gives off branches that join each of the peduncles. This ridge is highest and broadest opposite the suckers that are most modified and gradually disappears as the suckers become normal. At its highest point it has about the same elevation as the shortest modified suckers, which are adjacent. Sections of the modified portion of the arm show that the ridge and suckers mentioned are covered by a thick columnar epithelium that stains deeply. Many of these epithelial cells are filled with large rounded granules that stain with eosin. The cells that cover other portions of the arm are flattened or cubical, do not stain very deeply, and do not contain granules. It seems probable that the cells of the hectocotylized region secrete a substance that aids the arm in holding the spermatophores. The modified suckers probably make the bending and grasping necessary for the transfer of the spermatophores more easily accomplished.

The positions of the animals during copulation are rather hard to determine as the whole process generally does not occupy more than ten seconds and during this time the animals are usually swimming and the arms are changing positions, but by carefully focusing attention during different acts upon first one arm and then another, the positions and movements have been determined with some accuracy I think. Fig. 1 represents the positions of the animals while the arm of the male that bears the spermatophores is inserted into the mantle chamber of the female. This figure is the result of my conception of positions after having carefully observed copulation more than twenty times. Since drawing the figure many other observations have been made and the positions always seem to be essentially as given.

The male usually grasps the female while both are swimming. Occasionally the female may be resting on the bottom in the characteristic attitude, with the tips of the arms and the posterior end of the body touching and the head and funnel region somewhat elevated. If not swimming, she usually, when grasped, starts to swim, but in a few cases that I have observed she made no effort

and left the bottom only as she was lifted or turned by the male. In every case the male attached from the left side of the female. He frequently swims close to her and brushes the tips of his arms along her head and mantle. Just before attaching, if both are swimming, he sinks slightly beneath her and grasps her body with his arms so that his right arms are all on the right side of her body and his left arms are all on her left side. The body of the male is seldom exactly ventral to the female but usually slightly toward the left side. Attachment is evidently made as nearly as possible in the required position but when the female darts ahead, as she frequently does, the male is likely to attach too far posteriorly. In such cases he does not let go his hold but crawls rapidly forward, arm over arm, until the right position is attained. Naturally the positions of the individual arms differ somewhat but in general the arrangement is reasonably well shown in fig. 1.

For about a second after his position is attained the arms seem busy in making firm attachments, then with a rapid sweep his left ventral arm is passed by the end of his funnel and is immediately inserted into the mantle chamber along the left side of her neck, near the funnel. During the act both animals are usually quite without color and the inserted arm of the male may be seen fairly distinctly inside the mantle chamber.

The movement of the arm past the funnel is rapid and only once have I actually seen the grasping of the spermatophores and their transference to the mantle chamber. In this case while watching squid in an aquarium that was placed so the squid were between me and a window, a male grasped a female that was resting on the bottom. The female, contrary to the usual custom, did not move. As the male had attached far back on the body, opportunity was given me to get into position for observation before the male could crawl forward. As the female made no attempt to get free, the male seemed far more deliberate than usual. Just before the arm was passed by the end of the funnel, the penis could be seen protruding into it. A number of spermatophores appeared in the opening of the funnel and were grasped by bending the tip of the arm around them. With a rapid sweep of the arm they were immediately inserted into the mantle chamber of the female

where they were held about five or six seconds. The arm was then withdrawn and in about five or six seconds more the empty cases of the spermatophores passed out of the funnel of the female with a respiratory jet of water. These spermatophore cases were pretty closely attached to each other by having the tubes of their ejaculatory apparatus twisted together. They were recovered and found to be 41 in number. To the cluster were attached five sperm reservoirs. Examination of the female later showed that most of the other reservoirs were attached near the end of the oviduct. While the number of spermatophores used in an act of copulation varies greatly, the observations that have been made, indicate that this may be a little, but not much above the average.

The animals nearly always separate almost immediately after the arm is withdrawn. Beside the male which started to eat the female, a very few individuals have remained attached for from some seconds to nearly a minute after the arm has been withdrawn.

After copulation the female frequently seems considerably fatigued and may settle to the bottom and rest some minutes before becoming active again. I am rather inclined to think that this is due to her struggles, for when the female remained quiet, the apparent fatigue did not seem so marked. The male does not seem greatly affected, but is likely to continue to be very active for some time.

The copulation that leads to the filling of the sperm receptacle on the buccal membrane does not seem to be preceded by special movements. Although I have observed it several times the absence of preparatory movement has left me rather unprepared for the observations that must necessarily be made so quickly, for in this, as in the other form of copulation, the animals are seldom in contact more than ten seconds. In the cases I have observed my attention has been attracted by the sudden dart of one squid, the male, from one end of the aquarium directly at another, the female. Before the dart the squid face each other, and are separated by thirty centimeters or more. The movement was always exceedingly rapid and was probably due in each case to the

expulsion of a single jet of water. The male seemed to reach the female before she had time to move much, although she has given me the impression of attempting to dodge as if frightened. The two animals become attached head to head with their arms intermingled, each grasping the other (fig. 2). Then as in the other method, the male sweeps his left ventral arm past the end of the funnel and grasps the bundle of spermatophores. These are immediately thrust between the ventral arms of the female and held there for a few seconds. The animals then separate and examination has shown fresh sperm reservoirs attached to the receiving depression on the buccal membrane of the female. The empty cases of the ejaculated spermatophores may be held between the arms several minutes but they are finally dropped. Here, as in the other method of copulation, only the sperm reservoirs are retained for any length of time.

The spermatophores begin to ejaculate immediately after leaving the penis and the whole process is completed in a very few seconds. Pulling the filament attached to the ejaculatory end of a spermatophore is all that is needed to start its ejaculation. As the ejaculatory end of the spermatophore leaves the penis last and, as the spermatophores in the penis and the spermatophoric sac are imbedded in a viscid secretion, there is every reason to believe that the pull given the spermatophores by the arm with which they are grasped, when this arm starts to transfer them from the penis to the mantle chamber or to the buccal membrane, is sufficient to start ejaculation. The arm carries the spermatophores into the position necessary for the attachment of the sperm reservoirs while they are ejaculating and holds them there until the ejaculation is complete and the reservoirs are attached.

The structure of the spermatophores and the mechanics of ejaculation which lead to the attachment of the reservoirs will be treated in another paper. It should, however, be understood that the spermatophores are never attached as such, but they ejaculate and the sperm reservoirs are attached. As the reservoirs are attached by cement carried inside the spermatophores and liberated by the ejaculation, they may be stuck anywhere.

The sperm slowly escape from these reservoirs and may then

become free in the water, as when they are attached in the mantle chamber, or may be stored in a special receptacle, as when they are attached in the special depression on the outer buccal membrane. They are mixed with a viscid secretion in the reservoir and probably also before entering the reservoir, although I am not certain about the latter. The epithelium of the region is abundantly supplied with goblet cells which very possibly supply secretion for this purpose.

The depression in which the sperm reservoirs are mostly attached is supplied with a deeply staining columnar epithelium which is covered by a mass of rather hard material, evidently secreted by these cells, that shows distinct markings parallel with the surface of the epithelium (figs. 11 and 12). These markings seem to indicate that the material is secreted intermittently and thus is formed in layers. This material forms a suitable place for attachment of sperm reservoirs and probably serves no other purpose. Reservoirs are sometimes attached to other portions of the buccal membranes or to the tentacles but they are far more abundant in the depression than anywhere else. The sperm that escape from the reservoirs that are not attached in this depression probably do not find their way into the sperm receptacle.

The sperm receptacle has the shape of a compound alveolar gland (fig. 11). It is imbedded in the outer buccal membrane and opens on the inner surface of this membrane at a point opposite the junction of the two ventral arms. Simple cubical epithelium lines the deeper alveoli of the receptacle, and cubical epithelium with many goblet cells the portion nearer the opening. Some, but not many, cilia have been seen on these cells. The killing fluids used may not have preserved them, for the tails of sperm in the reservoirs are not often individually visible in the sections. With the exception of the tails of the sperm and the possible cilia on the cells the material gives evidence of good preservation. A layer of muscle fibers surrounds the receptacle as a whole and bundles of fibers run between and around the individual alveoli.

It was not determined whether the sperm are active in the interval between their discharge from the reservoirs and their en-

trance into the receptacle or not. That they are not active while stored in the receptacle is shown by opening filled receptacles on dry slides. The sperm are invariably quiet, but immediately become active when sea-water is added. In specimens killed soon after copulation, sections show the sperm entering the receptacle in narrow streams and not spread out as one might expect them to be if the sperm were active (fig. 11). It was not possible to remove all the sea-water from living specimens in which the receptacles were being filled without causing disturbances in the vicinity of the reservoirs and that made it impossible to determine the normal condition of the sperm in transit from one to the other. In the sections that show sperm entering the reservoir the tails all point in the same direction, as would be the case if they were not swimming actively but were being moved by an outside force. The heads go first and the tails all trail behind. Swimming sperm usually move in all directions but there may be some directive cause that would account for their positions even if they are stored through their own activities.

As previously stated, a female that is nearly ready to deposit her eggs can be told by her peculiar nervous movements and the way she manipulates her arms. Frequently the borders of the accessory nidamental glands, which are very red at this time, may be seen through the semi-transparent mantle and thus form a further indication that the eggs are nearly ready to be deposited. The nidamental and oviducal glands of such an animal are always somewhat, and frequently greatly, enlarged. Immediately after the eggs have been deposited these glands, while still large, are soft and flabby.

As is well known the squid deposits her eggs imbedded in strings of a jelly-like substance which vary in size with the size of the animal depositing them but which probably average about 8 mm. in diameter and 90 mm. long. The jelly consists of an inner mass that surrounds the eggs and a thick, rather tough but still jelly-like sheath that forms the outer covering. The inner jelly is secreted inside the oviduct by the oviducal glands. The outer jelly is secreted by the nidamental glands and is apparently moulded into shape as it passes through the funnel. The accessory nidamental

glands, which lie just in front of the anterior ends of the nidamental glands and open by wide openings near the narrow openings of these glands, are very active during this period and secrete a viscid material. What the special function of this secretion is has not been determined. It would seem from position and activity that the secretions from both sets of glands must be mixed as they are poured out.

Until recently eggs have not commonly been deposited in aquaria at Woods Hole. This may be due to the way the animals have been handled. Squid will not stand rough handling, either in capture or transportation, and live well in aquaria afterward. When captured in fish traps, quickly and carefully transferred to live cars where they are supplied with an abundance of water, and transported to the aquaria with as little excitement and as good water as possible, they may be kept several days in pretty good condition, but they wear themselves out by constantly bumping against the walls of the aquaria and are not vigorous many days. During each of the months I have worked at Woods Hole, June to late September, specimens have been obtained that have deposited eggs in aquaria. During the first three months specimens ready to deposit eggs are rather easy to get. In September only a small proportion of those captured still contained eggs.

Eggs are somewhat more frequently deposited in aquaria at night than during the day, but this may be due to the frequent if not nearly continuous disturbance to which they are subjected during the day in a laboratory where many people are working. The usual number of strings deposited by a female in what would seem to be a continuous laying period ranged from one to six. These strings were commonly delivered from fifteen to forty minutes apart, the time between any two strings being quite variable in an individual. One specimen, however, deposited twenty-three strings in an hour and thirty-five minutes. These were laid during a comparatively dark day when the laboratory was quiet. Possibly the small number deposited by other females was due to disturbance.

The end of the egg string begins to protrude from the end of the funnel while the female rests upon the bottom in the attitude

habitually assumed by resting squid (fig. 5). When from one to two centimeters of the egg string protrudes from the funnel, the female leaves the bottom and begins to swim slowly backward. This swimming is apparently due both to movements of the tail fin and to small jets of water forced from the funnel along the sides of the egg string. The jets of water cause the egg string to be protruded gradually. The protruding end is now caught by the ends of the two dorsal arms, which are bent ventrally between the other arms for this purpose (fig. 6), and as the string is ejected from the funnel, it is drawn between the circlet of arms. It usually takes from half a minute to a minute for the egg string to pass through the funnel and to disappear between the arms. It is then held between the arms about two minutes or sometimes a little longer. While the string is held between the arms it is completely enclosed by them and their free ends keep twisting around each other. In this position they form a cone with the apex at the ends of the arms (fig. 7). At other times the arms are held so they form a dorso-ventrally flattened expansion that serves somewhat as a rudder or anterior fin. The arms while enclosing the eggs are never entirely still but move slightly upon each other and are probably busy in moving the string about. While the string is thus held the animal slowly swims back and forth, never rapidly but continuously.

Toward the end of the period during which the string of eggs is held, the animal shows an increasing tendency to turn the body into a nearly perpendicular position to bring and keep the tips of the arms in contact with the bottom (left animal in fig. 9). With the arms held quite rigid and the tail fin moving rapidly she goes bounding along on the tips of her arms, dorsal side foremost, with a movement somewhat similar to the bounces that may be obtained by pushing a lead pencil, held by one extremity and slightly inclined from the perpendicular, over a table. This action is generally repeated several times. She occasionally catches hold of objects with her suckers, finally catches some object firmly, draws down into close contact with it for two or three seconds (right animal in fig. 9) and, when she releases her hold, leaves the string of eggs fastened to the object she had laid hold of. At

this time the jelly of the string is soft and sticky. It hardens quite rapidly and soon will not stick to objects, but at this time it adheres readily. The position of the string when taken between the arms indicates that the string is finally stuck by the end that first leaves the funnel.

After sticking a string of eggs the female rests upon the bottom some minutes before another string makes its appearance. She usually selects some protruding object like a stone, shell, or water pipe upon which to stick the egg strings. Having stuck one string she usually, but not always, returns to the same place to stick later strings. If strings are present when a female begins to deposit she usually attaches to these strings, or to nearby objects. This no doubt accounts for the very large clusters, with strings containing eggs in various stages of development, that are sometimes found. Upon several occasions clusters in fish-traps and live-cars have been found that would not go into an ordinary ten-quart pail. Such clusters are of course formed by many females.

It is evident that the eggs may be fertilized in the oviduct, in the mantle chamber, or between the arms. Examination of the contents of the oviduct have in no case given evidence of sperm. Eggs taken from the oviduct may easily be fertilized by placing them in sea-water containing sperm, but in no case did eggs taken from the oviduct show evidence of fertilization although many sperm reservoirs that were giving off active sperm were attached to the walls of the oviduct and to surrounding organs.

There can be no doubt, however, that eggs may be and are fertilized in the mantle chamber and also between the arms. That the eggs may be fertilized in the mantle chamber is indicated by reason rather than by observation. When sperm reservoirs are attached in the mantle chamber the sperm are constantly liberated in the water in this chamber as long as the supply lasts. The eggs upon leaving the oviduct also pass into the mantle chamber and, as before stated, when eggs and sperm are mixed in sea-water, fertilization results.

That fertilization may be delayed until the egg string is formed and held between the arms is indicated by observations made on

the female already mentioned that deposited twenty-three strings. She was in a rather large aquarium with a number of other squid. Copulation had occurred several times but this particular squid, which had been under observation some hours, had not been seen to copulate. Dissection later showed that there were no sperm reservoirs attached in her mantle chamber. Because of disturbance she upon six occasions failed to get the egg string between her arms. When she reached for the string with her dorsal arms she was each time disturbed so she dropped the string and ejected it directly into the water. Four of these strings were recovered as quickly as possible after they were dropped, and placed in dishes of fresh sea-water where the proportion of fertilized eggs could be determined. From 40 to 50 per cent of the eggs in the strings developed. More than 99 per cent of the eggs in strings that had been held between the arms and then placed in similar dishes developed. As already mentioned there had been copulation among other squid in the aquarium and as the reservoirs were attached in the mantle chambers there must have been many free sperm in the water of the aquarium. It seems probable that enough of these sperm reached the strings that were dropped, before they could be removed from the aquarium, to fertilize a portion of the eggs. Microscopic examination of these strings immediately after they were dropped revealed very few sperm, but the strings that were held between the arms were swarming with them. Sperm were able to penetrate and move actively about in the soft jelly of a recently formed string, but the jelly soon hardened so fresh sperm brought in contact with it were not able to work their way in.

A curious bit of habit reflex was exhibited by this squideach time she dropped a string of eggs. Immediately after the disturbance she took the attitude she would normally have taken had the egg string been successfully lodged between the arms. The arms were held in the form of a cone, the tips were twisted together and she passed on through each of the succeeding phases even to drawing down tight against an object as if to attach the egg string that had never been between the arms. After this she rested until the next string was formed, but she never interrupted the orderly

sequence of her activities because she had accidentally lost a string of eggs.

The methods of copulation of cephalopods have attracted the attention of observers from very early times but the act of copulation has not been actually seen for many species and where observations have been made they have for the most part been incomplete. Aristotle makes several statements regarding the breeding habits of cephalopods. It is quite possible that he saw something of the act of copulation for some species, but his statements are hard to follow and are evidently inaccurate. The most important statements are here quoted to show the curious medley of facts and fiction. In chapter 5, book 5, he says:

1. All the malacia, as the polypus, sepia and teuthis, approach each other in the same manner, for they are united mouth to mouth: the tentacula of one sex being adapted to those of the other; for when the polypus has fixed the part called the head upon the ground, it extends its tentacula which the other adapts to the expansion of its tentacula, and they make their acetabula answer together. And some persons say that the male has an organ like a penis in that one of its tentacula which contains the two largest acetabula. This organ is sinewy, as far as the middle of the tentaculum, and they say it is all inserted into the nostril of the female.

2. The sepia and loligo swim about coiled together in this way, and with their mouths and tentacula united, they swim in contrary directions to each other. They adapt the organ called the nostril of the male to the similar organ in the female; and the one swims forwards, and the other backwards. The ova of the female are produced in the part called the phyteter, by means of which some persons say that they copulate.

Again in chapter 10, book 5, he says:

1. The malacia breed in the spring, and first of all the marine sepia, though this one breeds at all seasons. It produces its ova in fifteen days. When the ova are extruded, the male follows, and ejects his ink upon them when they become hard. They go about in pairs. The male is more variegated than the female, and blacker on the back. The sexes of the polypus unite in the winter, the young are produced in the spring, when these creatures conceal themselves for two months. It produces an ovum like long hair, similar to the fruit of the white poplar. The fecund-

ity of this animal is very great, for a great number of young are produced from its ova. The male differs from the female in having a longer head, and the part of the tentaculum which the fishermen call the penis is white. It incubates upon the ova it produces, so that it becomes out of condition, and is not sought after at this season.

Part of these statements, such as "The sepia and loligo swim about coiled together in this way, and with their mouths and tentacula united, they swim in contrary directions to each other" would seem to be based upon such observations as could be made from above but the further statement that they adapt their nostrils (funnels) together, probably indicates the ease with which observation and supposition can be mixed. It is not necessary further to analyze Aristotle's statements. No doubt much was based upon fishermen's stories but he evidently did study the anatomy and habits of these animals and recognized the probability that one of the arms of the male is used in copulation.

While the modified arm of the male thus early received attention, the true hectocotylus that separates entirely from the male and attaches itself in the mantle chamber of the female escaped notice for many centuries. To quote from the Cambridge Natural History:

The typical hectocotylus seems to have entirely escaped notice until early in the present (last) century, when both Delle Chiaje and Cuvier described it, as detected within the female, as a *parasite*, the latter under the name of *Hectocotylus octopodis*. Kölliker, in 1845-49 regarded the *Hectocotylus* of *Tremoctopus* as the entire male animal, and went so far as to discern in it an intestine, heart, and reproductive system. It was not until 1851 that the investigation of Vérany and Filippi confirmed a suggestion of Dujardin, while H. Müller in 1853 completed the discovery by describing the entire male as *Argonauta*.

While nearly all male cephalopods show some modification of one or more arms, the only ones that have been reported with detachable arms are *Argonauta*, *Ocythoe*, and *Tremoctopus*.

Extended studies have been made on the modification of the arms of cephalopods, and there have been a few observations upon

the functional activities of these arms, but most of the observations have consisted in finding sperm reservoirs recently attached to various portions of females.

In 1869 Lafont described copulation in *Sepia*. A translation of that portion that deals with the act itself is as follows:

In copulation the male and female precipitate themselves upon one another, hold together by their arms which are twined together, and remain thus, mouth to mouth, for a variable time, which may last for two or three minutes. This act is followed in the female by a state of very marked general prostration, while in the case of the male the general excitation is greatly prolonged and for a considerable time it keeps the splendid appearance these animals show as the result of the accomplishment of the function of reproduction.

He supposed that while the animals were attached by their arms, head to head, the male ejected a packet of spermatophores, which ejaculated while in his mantle chamber and the sperm reservoirs were then thrown from the funnel of the male into the branchial chamber of the female with the current of water entering her branchial chamber.

*Sepia*, like *Loligo*, has a receptacle for the storage of spermatozoa in the buccal membrane, and the position observed by Lafont of animals attached head to head was doubtless a true position of copulation, but it seems probable that the spermatophores were not disposed of in the way suggested, but were transferred to the buccal membrane by one of the arms of the male. Lafont found sperm reservoirs attached in the mantle chamber of the females near the mouths of the oviducts, so it seems probable that in this form, as in *Loligo pealii*, both methods of copulation occur.

Racovitza (1894, a) observed copulation in *Sepiola*. The male seized the female, turned it over and inserted his first pair of arms into the mantle chamber. Copulation lasted eight minutes during which the female struggled to free herself. He speaks of the spermatophores being fixed on the folds of a large pocket situated on the left side of the pallial cavity of the female. These ejaculate and the freed reservoirs deliver their sperm into the pocket

which in turn ejects them (from his description I take it they are not stored up in this pocket as in the receptacle on the buccal membrane of a squid) into the pallial cavity where they are supposed to meet the eggs as they are laid.

The most complete account of copulation that I have seen for any cephalopod was given by Racovitza in 1894 (b) for *Octopus vulgaris*. He observed copulation in an aquarium and gives a figure showing the positions of the animals. The copulation differs markedly from that of *Loligo*, as might be expected, for *Octopus* has a hectocotylyzed arm that is much more differentiated than that of *Loligo*. The animals were some distance apart in the aquarium. The male reached over with the hectocotylyzed arm, which for this species is the third on the right side and, after caressing the female with its tip, introduced its end into her mantle chamber by the side of the funnel. Here it remained for something more than an hour. During this time the female remained quiet, except for certain spasmodic movements, while the male showed only slight movements of the hectocotylyzed arm which were supposed to be associated with the movements of spermatophores down the longitudinal groove of this arm. Although it was not possible actually to see the spermatophores in transit, examination of the female after copulation showed numbers of the sperm reservoirs, derived from the ejaculated spermatophores, within the oviducts.

Evidently there are at least three methods of copulation practiced by cephalopods. A method of caducous hectocotylysm in which the charged hectocotyl is liberated in the mantle chamber of the female; a method in which the arm does not liberate any special portion but is so modified that it can transfer spermatophores by a mechanism within itself to the region of the oviduct of the female; and finally a slight modification of the arm that simply enables it to grasp the spermatophores which are then transferred directly to the female by moving the arm. Where the latter method is employed there may be two kinds of copulation, as in *Loligo pealii*.

Racovitza, (1894, c) in commenting on the copulation of *Rossia* believes that, although special receptacles are found outside the

mantle chamber of this species, they cannot be considered as normally functional. He seems led to this conclusion by finding sperm reservoirs attached to various portions of the bodies of the animals as well as in the immediate neighborhood of the mouths of the oviducts. It would seem more likely in the light of the observations here recorded for *Loligo*, that a copulation that leads to the filling of these receptacles is normal and that the sperm so stored may be used in fertilizing the eggs.

It is certainly hard to conceive by what steps a complicated method of transferring sperm that has led to the formation of a hectocotylyzed arm and complicated spermatophores might be perfected. The modification of different arms for copulation by different cephalopods further increases the difficulty in understanding the history of hectocotylysm as a whole.

While evidence that bears directly upon the history of the hectocotylysm seems to be lacking, such complications are so frequently considered to be impossible to explain by known evolutionary factors that it may be well at least to consider the great difficulties presented by such structures. It must not be supposed that in so doing I put myself in the position of defending a thesis. This would be too much like the methods employed by many of the Greek philosophers who needed little or no basis of fact upon which to build. My only reason for considering the matter here is to show that, with all the difficulties, the condition of hectocotylysm among modern cephalopods cannot be considered beyond the possible range of evolutionary factors.

Among the Dibranchiata the arms that show hectocotylysm are the first, the third and the fourth on both sides of the body. Sometimes more than a single one is affected. In such cases the modified arms may be symmetrically placed on the two sides of the body, or they may be adjacent arms on the same side of the body. Steenstrup attempted to base the classification of cephalopods upon their hectocotylyzed arms but Brock and Hoyle have shown that forms whose general body structure would seem to indicate relationship, do not always have homologous arms modified.

While the arm is usually constantly on one side for all members of a genus, unless both sides are modified as not infrequently hap-

pens, a genus whose general body structure indicates nearrelationship may have the similar arm of the other side modified. The position of the arm on the right or left side of the body is not generally considered very significant. The somewhat frequent occurrence of genera showing hectocotylism of arms symmetrically placed on the two sides of the body may indicate a primitive paired condition that has been replaced among the majority of existing cephalopod genera, by specializing on one side and dropping out on the other. Whether this accounts for the condition or not, the shifting of a modification from one side of the body to the other, sometimes involving modifications of other body structures and sometimes apparently not, is not uncommon among animals, and even if not easily explained, evidently has no very great phylogenetic significance. Shifting in series is not so common and when we find in the same family, genera with the fourth and others with the first arm hectocotylized it becomes difficult to imagine ancestral conditions that made this possible.

Wherever known, male cephalopods use one or more of the arms to transfer sperm to the female. Copulation has not been described for many of the species but the presence of more or less modified arms in more than half the recognized families may be taken as an indication that either these animals or their ancestors used their arms in copulation.

If the spadix of *Nautilus* is used in copulation we have a possible indication that a number of arms may have been employed in the transfer of sperm by primitive cephalopods. It is of course possible that all the arms were used for this purpose and that the present diversity can be accounted for by the specialization of one or the other of the arms involved in this primitive condition. This, however, does not seem reasonable when the diversity within the limits of a single family is considered.

The arm that is used, and the way in which it is used, is associated with the character of the spermatophores and the position of their final discharge. The Octopoda show the greatest structural modification in their hectocotylized arms. While two of the families of this group give no evidence of hectocotylism, none of the genera of the remaining families are known to be free from it,

and wherever found it is always the third arm that is involved. Sometimes this arm is on the right and sometimes it is on the left side. In three genera it is known to be caducous and in a fourth (*Alloposus*) it is supposed to be. In the remaining genera in which the hectocotylyzed arm has been studied, the modifications, while not resulting in the actual separation of the arms, are of an extensive nature. In *Octopus*, for instance, they involve not only changes in size, form, and the condition of suckers, but a special groove is present through which the spermatophores are supposed to be carried from the base, presumably from the penis to the tip. The tip in turn is modified so it is supposed to function in placing the spermatophores in position for ejaculation.

The Decapoda do not show such extensively modified hectocotylyzed arms. The changes are here chiefly confined to some of the suckers and their immediate vicinity. In *Loligo* this modification apparently serves to aid the arm in grasping the spermatophores, which are then transferred by the movement of the arm. While the actual grasping of the spermatophores has not been previously observed, there can be little doubt that other forms of the Decapoda use the arms in a similar manner. Where copulation has been observed the movements of the arms indicate that they are used in the transfer, and the positions of the sperm reservoirs that have been found attached to the females indicate that some arm must have functioned in getting them into position. As there is no special transferring mechanism, this must have been accomplished by the free movements of the arms.

Where structural modification is slight and the placing of the spermatophores is due to dexterity, there is less difficulty in understanding how the function may be shifted from one arm to another in response to changes in the position of the attachment of the reservoirs on the female, than would be the case were great structural changes involved. It would be much more difficult to understand how there could be a shifting in series of arms as highly modified as those of the Octopoda, where only the modified arm could possibly perform the function.

It must not be understood that habit formation requiring such dexterity is considered easier to originate than modification in

structure that will perform similar acts. When, however, the habit and dexterity have been acquired, it is not inconceivable that they might be shifted to another closely similar appendage if the position of this appendage becomes more suitable for the purpose. The modification is so slight in the arms of most of the Decapoda, and the modification varies so greatly in the different genera, that it may have been functionally acquired in each case. So far as can be seen it would be mechanically quite possible for a squid to use an unmodified arm, instead of the one that shows the modification, for the transfer of the spermatophores. The spermatophores might not be so tightly or compactly held but the normal suckers would hardly seem to interfere greatly in the performance of the function.

There is still another question involved. Is there any genetic relation between these two methods of transfer and if there be, which, if either, most probably came first?

A special method of copulation that requires the use of arms and complicated spermatophores is not found among animals often enough to make it at all probable that it has arisen in this group more than once, so we can hardly doubt that the two methods are genetically related.

At first sight the squid's method of grasping the spermatophores and transferring them directly might be considered the simpler process, but there is some reason to doubt that this method was at the beginning of the series. While it would be hazardous to say that Octopoda were the ancestors of Decapoda, there is much reason to believe that the ancestors of the latter lived upon the bottom and were far less active than the modern animals. Such animals would not seem to be so well adapted for the transfer of spermatophores by dexterous movements as the more active, free-swimming forms. It is at least certainly true among modern cephalopods that those that show great modifications in the structure of the hectocotylized arms are found entirely among the less active bottom forms. If the method of transferring sperm by means of the arms originated before the Decapoda became free-swimming animals, and this seems the only explanation of its prevalence

among both Decapoda and Octopoda in modern times, it would seem that structural modification probably came early.

Possibly this modification was based upon the use of one or more arms as guides for the transfer of the sperm. It is possible that having first used the arms as guides, structural modifications and dexterous movements were developed as divergent methods. If the two methods form a linear series, there is some reason to think structural modifications came first. It would seem much easier to explain modifications that lead to the change in the structure of appendages for the transfer of spermatozoa, as the grooved hectocotylized arm of *Octopus* or the modified abdominal appendages of certain Crustacea, than to explain a sudden change that would result in a practically unmodified arm functioning by grasping spermatophores of a very specialized kind, transferring them quickly and accurately to the required position and holding them there until they have had ample time to ejaculate and fix their contents. It seems more reasonable to suppose that an arm modified as a machine to perform this process, with its tip serving to place the spermatophores in position, might in time acquire the necessary dexterity and then lose the modifications previously acquired, than to look at this as the beginning of the series. Again we find that in such cases as the squid, where the arm is little modified but very dexterous, there is a special receptacle at some distance from the opening of the oviduct that is normally filled with sperm during the breeding season. This would certainly seem to be a comparatively recently acquired receptacle, so the copulation leading to its being filled would also be considered comparatively recent. That this receptacle is concerned in the fertilization of the eggs is shown by observations made while the eggs were being laid.

With no personal knowledge of the breeding habits of other cephalopods than the squid, it would seem more reasonable to consider the method of using the detachable hectocotyl of such forms as *Tremoctopus* as one extreme, the method used by *Loligo* in grasping spermatophores and transferring them directly as another extreme and the condition shown by *Octopus* as the modern greatly specialized product of a modification such as early cephal-

opods probably developed. This would mean that the detachable hectocotyl is an extreme specialization in structure and that the modification shown by the squid represents possibly a degeneration in structure but a remarkable specialization in habit.

Why a form should have two methods of copulation is not at all clear. Certainly the introduction of the spermatophores into the mantle chamber to a position near the oviduct is to be considered more primitive than their being placed in a position to fill a receptacle outside of the mantle chamber, but why mantle chamber copulation should be retained after the receptacle has been perfected is not clear. That mantle chamber copulation is not absolutely necessary for the fertilization of the eggs I think is proved by my observations; that it is common is certain. That the sperm receptacle is an improvement over the free attachment of the sperm reservoirs in the mantle chamber is evident from the longer possible retention of the sperm in the receptacle. In a limited period after the sperm reservoirs are freed from the spermatophores, as when deposited in the mantle chamber, the sperm all escape and are wasted unless oviposition takes place in the meantime.

#### SUMMARY

Squid have two methods of copulation. By one method sperm reservoirs are attached in the mantle chamber on or near the oviduct and immediately begin to discharge their contents freely in the water. By the other method sperm reservoirs are attached to the outer buccal membrane and the sperm become stored in a special receptacle in the membrane, which is placed opposite the junction of the two ventral arms and opens on its inner surface.

The left ventral arm of the male is always used in transferring the spermatophores, which are grasped by the arm and transferred by its free movement. Ejaculation of the spermatophores is evidently started by the pull given their filaments when the arm starts to transfer them from the penis to the mantle chamber or buccal membrane. The transfer requires rapidity and dexterity and the spermatophores are held in position until ejaculation is complete and their sperm reservoirs are fastened. As many as forty spermatophores may be transferred at a time.

The egg strings are composed of two kinds of jelly. One kind is supplied by the oviducal gland and the other by the nidamental and probably accessory nidamental glands. The string is apparently molded into shape by passing through the funnel. The jelly is at first soft and sticky but soon becomes tough and loses most of its stickiness.

From the funnel the egg string is drawn between the circlet of arms, where it is held two or more minutes. In sticking the string the female grasps some object with her arms and draws down tight so the string is evidently crowded against it. When she releases her hold the string is left sticking to the object.

Fertilization evidently does not take place inside the oviduct. It doubtless may take place in the mantle chamber when sperm reservoirs are present there, and is known to take place while the egg string is held between the arms. The sperm are liberated from the receptacle while the eggs are between the arms.

Notwithstanding complications, the conditions of hectocotylism shown by cephalopods need not be considered beyond the influence of factors of evolution.

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## EXPLANATION OF FIGURES

All of the figures that represent the attitudes of squid were drawn from memory after repeated observations. While each figure is thus really a composite, and must represent impressions received rather than the actual positions of particular individuals, much care has been given to the preparation of the figures and it is believed that the general attitudes are reasonably well represented. Sexually mature squid are usually as much as 15 cm. and may exceed 40 cm. in length.

## ABBREVIATIONS

<i>bmi</i> , inner buccal membrane	<i>n</i> , nidamental gland
<i>bmo</i> , outer buccal membrane	<i>na</i> , accessory nidamental gland
<i>d</i> , depression in which sperm reservoirs are attached	<i>o</i> , oviduct
<i>g</i> , gill	<i>r</i> , rectum
<i>h</i> , modified (hectocotyized) portion of arm	<i>s</i> , sperm reservoirs (ejaculated from spermatophores)
<i>j</i> , jaws	<i>sr</i> , sperm receptacle
	<i>sro</i> , opening of sperm receptacle

## PLATE 1

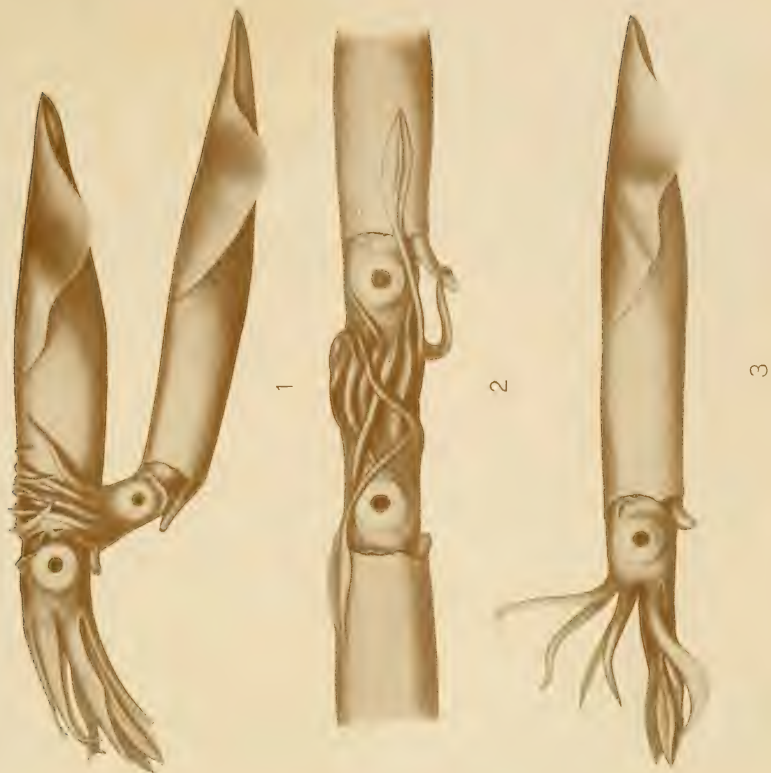
## EXPLANATION OF FIGURES

1 Copulating squid showing the positions taken by the animals when the spermatophores are inserted into the mantle chamber. The figure shows the animals during the period the arm of the male is inserted in the mantle chamber of the female. Drawn from memory after many observations.

2 Copulating squid showing the positions of the animals when the spermatophores are placed so that their reservoirs become attached to the outer buccal membrane. The figure shows the male in the act of grasping the spermatophores with the tip of his arm as they are ejected through the funnel. Drawn from memory after many observations.

3 A common attitude of a sexually excited male. The arms are not kept rigidly in a set position, but are frequently spread as shown in the illustration and held thus for from a few seconds to a minute or more at a time. The drawing is based upon sketches made of active animals.

4 Photograph of the two ventral arms of a male squid, showing the slight modification (*h*) consisting of enlarged peduncles, reduced sucking discs and a ridge between the suckers, toward the tip of the left arm. The wrinkles on the arms are due to shrinkage. A bit of the outer buccal membrane shows between the arms. The arms from which the photograph was made are  $9\frac{1}{2}$  cm. long.



Drew, del.



## PLATE 2

### EXPLANATION OF FIGURES

5 A female at rest with the egg string beginning to protrude. Drawn from memory and hurried sketches after many observations.

6 A female after she starts to swim, reaching for the egg string with her dorsal arms. With these arms she draws the string between the circle of arms as it is ejected from the funnel. Drawn from memory after many observations.

7 A swimming female, showing the positions of the arms while they surround the egg string. They are held in this position, with the tips somewhat twisted together, for two or three minutes. While the arms closely surround the egg string they show slight individual movements that may be of service in moving the egg string so sperm will be more evenly distributed over it. Drawn from memory after many observations.

8 A female squid with the mantle cut and spread open and the arms separated to show the position of attached sperm reservoirs (*s*) on the oviduct (*o*) and the sperm receptacle (*sr*) in the outer buccal membrane.



5



6



7



8

### PLATE 3

#### EXPLANATION OF FIGURE

9 The specimen on the left side shows a female in the position she assumes as she bounces over the bottom on the tips of her arms just previous to selecting a place for sticking the egg string. The specimen on the right side shows the position of a female during the act of sticking an egg string to a rock. Only a few seconds are required to stick the string. The positions of the animals are drawn from memory after many observations.



## PLATE 4

### EXPLANATION OF FIGURES

10 Jaws and buccal membrane of a female squid, with the outer membrane (*bmo*) pulled ventrally to expose the sperm receptacle (the opening of which is shown at *sro*) and the adjacent depression (*d*). Several sperm reservoirs (*s*), ejaculated from spermatophores, are shown attached in the depression. Magnified about 7 diameters.

11 Section of the outer buccal membrane taken through the sperm receptacle (*sr*). This was taken from an animal shortly after the sperm reservoirs (*s*) had been attached and shows sperm in transit from reservoirs to receptacle. Magnified about 22 diameters.

12 Section through the epithelium and secretion lining the depression in which sperm reservoirs are attached. Magnified about 300 diameters.

13 Section through an alveolus of a sperm receptacle. The clear spaces in the epithelium are goblet cells. Traces of the flagella on the sperm and possibly cilia on some of the epithelial cells were visible but they were not definite enough to be put in the drawing. Magnified about 300 diameters.



11



10



12



13



# STUDIES OF FERTILIZATION IN NEREIS

## I. THE CORTICAL CHANGES IN THE EGG: II. PARTIAL FERTILIZATION

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TEN FIGURES

ONE PLATE

### I. THE CORTICAL CHANGES IN THE EGG

In many animals one of the immediate effects of fertilization is to cause the egg to throw off a membrane, which is therefore known as the fertilization membrane. This is the case for instance in the eggs of echinids and nematodes. In other cases, where a definite vitelline membrane exists prior to fertilization, cortical changes occur in the egg immediately after insemination, leading to the formation of a space, the so-called perivitelline space, between the protoplasm of the egg and the vitelline membrane. This is the case for instance in the eggs of at least many annelids, molluscs and vertebrates, and it is unquestionably a more common phenomenon than the formation of a fertilization membrane. There can be little doubt that these apparently different phenomena are simply varying expressions of a change in the cortex of the egg, which is of the same nature in all cases. Loeb's studies ('09) have thrown much light on the nature of these cortical changes. In the case of the egg of *Nereis* they are relatively obvious in their character and readily followed.

The ovocyte of *Nereis* is somewhat flattened in a polar direction, measuring about  $87.5 \times 100\mu$ ; it is girdled by a double equatorial zone of large oil drops. The large germinal vesicle is central and somewhat elongated in a polar direction.

In his description of the unfertilized egg, Wilson ('92) distinguished two membranes: a delicate outer vitelline membrane,

and a subjacent membrane or layer, about  $6-7\mu$  in thickness, which he called the zona radiata. As will appear from the sequel however, the latter is not a membrane in the usual meaning of the word, but a cortical, coarsely alveolar layer of the egg. It is transparent and somewhat granular, and the granules tend to be arranged in radiating lines. There is no perivitelline space in the unfertilized egg.

In sections of unfertilized ovocytes fixed in Flemming's fluid, the zona radiata is seen to be a coarsely alveolar layer with homogenous alveolar contents (fig. 1). The walls of the alveoli are continuous internally with the protoplasm of the egg, and unite externally to form a protoplasmic layer applied to the vitelline membrane. The alveoli are closed externally (figs. 1 and 2). The zona radiata is in fact a coarse emulsion or foam-structure.

Unfertilized eggs of *Nereis* are entirely devoid of jelly and they lie in immediate contact in the sea-water. If India ink be ground up in the water, the particles come in contact with the vitelline membrane. Each *fertilized* egg, on the other hand, is surrounded by a thick layer of colorless transparent jelly; If many eggs are contained in the dish, fusion of the contiguous gelatinous membranes binds the eggs into a mass; the cortical layer (zona radiata) is absent in fertilized eggs, and there is a narrow perivitelline space between the vitelline membrane and the surface of the egg (fig. 3).

The jelly is formed by the extrusion, or diffusion, of the alveolar contents of the cortical layer through the vitelline membrane; the egg of *Nereis*, in fact, secretes its own jelly, as may be readily demonstrated in life by inseminating under the microscope with excess of sperm. If the sperm be added to closely placed eggs and a cover glass applied so as to force the eggs into a single layer, and the preparation examined with no loss of time, the spermatozoa will be seen in large numbers in contact with the vitelline membrane. In one or two minutes the spermatozoa are moved away from the surface of the membrane by some invisible repelling substance, and, if the eggs be numerous, the spermatozoa unite in three to five minutes to form lines that bound hexa-

gonal areas with the eggs in the centers of the hexagons (fig. A). The substance that sweeps the spermatozoa away from the surface of the eggs is the jelly. Synchronously with its formation, the alveoli of the cortical layer are emptied and the alveolar walls now appear as delicate lines crossing a wide perivitelline space<sup>1</sup> (fig. B).

However, not all of the spermatozoa are thus carried out by the secreted jelly, but in the case of each egg a single spermatozoon remains attached to the vitelline membrane. This is very prettily demonstrated if the eggs are under some pressure, so that

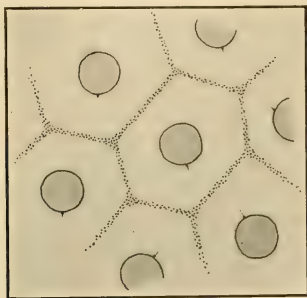


Fig. A. Diagram of fertilization with excess of sperm. The outflow of jelly from the eggs has carried the supernumerary spermatozoa away from the surface of the eggs (see text). In the case of each egg the single effective spermatozoon remains attached. From a sketch of the living object.

the spermatozoa are prevented from reaching the eggs above or below. In this case one can discover the single spermatozoon attached to the vitelline membrane in practically every egg (fig. A).

All stages of the disappearance of the cortical layer may be readily and rapidly observed. The alveolar walls, however,

<sup>1</sup>Wilson ('92) states that "from twenty to thirty minutes after fertilization the striae of the zona suddenly become indistinct and in the course of two or three minutes the zona itself entirely disappears, leaving only the outer membrane." But inasmuch as he was under the impression that the unfertilized eggs possess a transparent, thick, gelatinous envelope like the fertilized ones, he failed to observe the interesting phenomenon of formation of the jelly described here.

remain as delicate strands of protoplasm uniting the vitelline membrane to the surface of the egg. The jelly, therefore, represents the alveolar contents only of the cortical layer of the unfertilized egg, and the perivitelline space is nothing but the contracted alveoli of the cortical layer filled with fluid. The perivitelline space must, therefore, be regarded as *intraovular* with a delicate external cytoplasmic wall lining the vitelline membrane; this we may distinguish as the *plasma membrane*; it is comparable in some respects to the fertilization membrane of sea-urchins.

Unfertilized eggs allowed to stand in the sea-water form no jelly and retain the cortical layer; the germinal vesicle remains intact; but, if they be strongly centrifuged, the jelly forms, the perivitelline space arises, the germinal vesicle breaks down and both polar bodies are formed; but parthenogenetic development, usually at least, does not take place. Similarly, the addition of a fairly strong solution of potassium chloride to the sea-water causes formation of the jelly and the perivitelline space while the eggs are still in the solution; maturation takes place after transfer to sea-water but cleavage does not occur (in my experiments; cf. Fischer), though some differentiation may take place without cleavage. It would appear, then, that conditions that so alter the permeability of the plasma and the vitelline membranes as to permit the outflow of the alveolar contents of the cortical layer initiate development, but that the normal continuation of development is dependent on other factors.

In the normal fertilization of *Nereis* the stimulus of the spermatozoon causes the formation of the jelly and the perivitelline space long before it has penetrated the membrane; in fact penetration does not take place until 40 to 50 minutes after insemination. However, mere *contact* of the spermatozoon with the membrane is apparently not sufficient; but actual *attachment* of at least a single spermatozoon is required; this is shown by the fact that the effective spermatozoon is not carried away from the membrane with the unsuccessful ones by the outflow of jelly. Yet the effect of the localized stimulus of the attached spermatozoon is practically instantaneously effective over the entire

extent of the membrane; it is more like an electrical discharge or some other physical disturbance than a chemical effect.

J. Loeb ('09) has formed the hypothesis that the cortical layer of the egg, especially of sea-urchins, is an emulsion which is rendered stable by a third substance consisting of lipoids, especially cholesterin. The emulsion becomes unstable on solution of the lipoids; this enables the albuminous drops, which he conceives to form one phase of the emulsion, to take up water; hence the layer liquefies and the perivitelline space arises; the fertilization membrane is thus formed. Hence, according to Loeb, the action of lipid-dissolving substances is to cause the formation of the fertilization membrane. Without committing ourselves to these specific views of the nature of the cortical emulsion, which Loeb himself does not hold very strongly, we may admit that Loeb's hypothesis, that the formation of the fertilization membrane is due to the breaking down of a cortical emulsion, fits the case of *Nereis* very well. If we go further, however, we must note an important lack of agreement with Loeb's hypothesis. As Loeb himself points out, the theory implies that the membrane of the egg is permeable for sea-water and crystalloid substances, and on the other hand impermeable for colloids; in *Nereis* the contents of the cortical alveoli are unquestionably colloid, as Loeb's hypothesis requires, but it is perfectly certain that they diffuse through the membrane to form the external jelly; at the same time, unquestionably, sea-water enters to take the space previously occupied by the colloid. The membrane is therefore permeable for both crystalloids and colloids at this time. I have not, however, investigated farther the properties of the egg membranes and must leave this problem to those who are better qualified as physiologists to make such a study.

It would appear that the presence of this colloid substance in the cortex is an inhibition to the maturation of the egg, because as soon as it is removed, maturation processes are set in motion and both polar bodies are formed. In what manner it inhibits is of course problematical. In the egg of *Ascaris megalocephala* there is a similar excretion of a cortical colloid which forms, in this case, the thick resistant perivitelline membrane. The ap-

pearance of the fertilization membrane of echinids might be similarly due to excretion of a cortical colloid which is removed by diffusion and hence is not detected. It is a problem worthy of careful investigation whether the loss of cortical colloids is not the first step in fertilization generally.

## II. PARTIAL FERTILIZATION

Two functions of the spermatozoon in fertilization may be sharply distinguished. The first is the initiation of the development and the second is the transfer of paternal qualities to the fertilized ovum (heredity from male parent). The first function alone is under consideration in these experiments.

We have seen that in *Nereis* the immediate effect of attachment of the spermatozoon is essentially the same as a mechanical shock (centrifuging), or a chemical stimulus (KCl); that is, it causes the breaking down of the cortical emulsion and consequent formation of the gelatinous envelope of the egg. But apparently the resemblance extends no farther, for in the case of mechanical or chemical stimulation the impulse to development is lost or greatly weakened after maturation has occurred; and the eggs do not segment. On the other hand the normally fertilized egg does not stop after maturation, but proceeds with its development in a normal fashion. Now the cause of this difference might be either: (a) because the stimulus of the spermatozoon is qualitatively different from, or stronger than, mechanical or chemical stimulation, or (b) because the fertilizing action of the spermatozoon is not completed with the cortical changes but continues after its entrance into the egg. If the first alternative were correct, then the elimination of the spermatozoon after membrane formation should not prevent the normal cleavage and development of ova which had once been stimulated by it; but if the second alternative were correct and the sperm nucleus were prevented from entering the egg after it had induced membrane-formation, then such ova should proceed no further in their development than those mechanically or chemically stimulated.

I have been able to perform this experiment on the eggs of *Nereis* and have found that eggs in which the spermatozoon is removed after the cortical changes have occurred proceed but little farther in their development than eggs mechanically or chemically stimulated, and they do not undergo segmentation. Fertilization is therefore still incomplete after the formation of the fertilization membrane.

It will be seen that if the results above indicated be demonstrated, the process of fertilization is obviously something more than a beginning of cytolysis or a mere alteration of permeability of the peripheral cell membrane. It would appear to be a progressive change, starting at the periphery and gradually involving the more central portions of the cell. We would, at least, have to distinguish two stages in the fertilizing action of the spermatozoon, one before and the other after penetration.

I shall consider first the evidence for the statement that in the egg of *Nereis* elimination of the spermatozoon after membrane-formation leaves the process of fertilization incomplete. In the second place I shall note the respects in which the completely fertilized egg differs from the partially fertilized egg, and finally, shall consider the bearing of the facts on the theory of fertilization. Inasmuch as it will be necessary to make frequent comparisons with the normal fertilization, a brief account of the salient features of this process will be given first.

#### *A. Salient features of the normal fertilization*

The egg of *Nereis* is difficult to fix in a thoroughly satisfactory fashion; owing, no doubt, to the presence of the large oil-drops and yolk-granules, uneven fixation with shrinkage is hard to avoid. The eggs appear likewise difficult of penetration, owing probably to the rather viscid jelly from which they cannot be separated; this also makes any considerable number of eggs very bulky and the killing fluid is apt to be much diluted if used in ordinary amounts. After considerable experimenting with picric acid, corrosive sublimate and osmic acid fixing fluids, I finally found one which gives practically perfect results in all

stages of maturation and fertilization. This is Meves' modification of Flemming's fluid made as follows: chromic acid, 0.5 per cent, 15 cc.; osmic acid, 2 per cent, 3.5 cc.; glacial acetic acid 3 drops. The eggs were left in the fluid from thirty to forty-five minutes. Fixation in this fluid causes no shrinkage, the oil is so changed that it is not dissolved out by subsequent imbedding in paraffine; the sections stain beautifully in iron haematoxylin, and certain substances are clearly differentiated which can be detected only with the greatest difficulty after fixation in any other fluid tried.

a. *The penetration of the spermatozoon.* It was noted in the first part of this paper that a single spermatozoon becomes attached to the egg-membrane immediately after insemination, and that the breaking down of the cortical layer, secretion of the jelly and formation of the perivitelline space follow immediately, though the actual penetration of the spermatozoon is delayed forty or fifty minutes.

The act of penetration involves no motile activity on the part of the spermatozoon; after the latter has become attached to the vitelline membrane all movement of the spermatozoon ceases and it remains absolutely immotile throughout the forty or fifty minutes that elapse before it is taken into the egg. The events of this period as seen in the living egg are as follows:

1. The jelly is formed by outflow of the alveolar contents of the cortical layer-as already noted; although a large amount of jelly is formed in two or three minutes, yet the process lasts ten or fifteen minutes before the deeper alveoli are emptied. There is then a very wide perivitelline space crossed by the alveolar walls which are attached to the plasma membrane, presenting a very striking appearance (fig. B).

2. The protoplasm of the egg immediately beneath the attached spermatozoon then forms a rounded elevation, the entrance cone, which gradually moves across the perivitelline space and comes in contact, and fuses, with the membrane (fig. B, a). This condition is usually fully attained about fifteen to seventeen minutes after insemination.

3. The entrance cone then gradually retracts, drawing the membrane down to form a depression in which the spermatozoon is included. At this stage one may easily imagine that the sper-

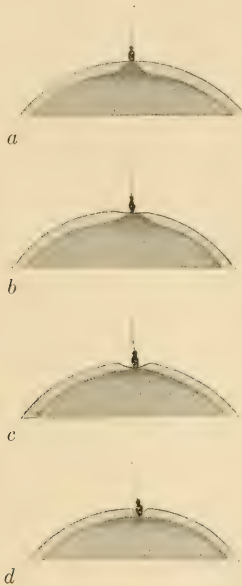


Fig. B. History of the fertilization-cone as seen in the living egg. Four camera drawings of the same egg:—

- a* Seventeen minutes after insemination,
- b* Nineteen minutes after insemination,
- c* Twenty-two minutes after insemination,
- d* Twenty-four minutes after insemination,

The fertilization-cone is shown at the height of its development in *a*, its gradual recession and the simultaneous formation of a depression in the membrane is shown in *b*, *c* and *d*.

matozoon has been taken into the egg, as it is apt to be concealed in the depression of the membrane; but this is not the case. The stage of best development of the depression, corresponding to

the complete retraction of the entrance cone, is about twenty-two to twenty-five minutes after fertilization (fig. B, b, c, d).

4. The perivitelline space then narrows around the entire egg, and the depression of the membrane in which the spermatozoon is seated disappears; in consequence, the spermatozoon again becomes prominent externally.

5. It remains prominent for ten or fifteen minutes (about forty to fifty minutes after insemination), and then disappears rather abruptly within the egg as though some resistance had given away. Its penetration coincides with the late anaphase of the first maturation division; in a few cases it may be a little earlier or a little later.

The egg is changing form at this time and in consequence the perivitelline space is often widened locally, especially in the animal hemisphere; if this happen in the region of penetration, which may be any part of the egg, strong cytoplasmic strands are drawn out between the membrane of the egg and the point of penetration, showing that the egg membrane and the cytoplasm are actually fused here.

To repeat and extend the observations on the living egg several series of eggs were preserved every five minutes from the time of insemination in Meves' fluid. The study of these sections confirmed and extended the above observation on the living egg as follows:

Ten minutes after insemination the entrance cone is quite well formed and the spermatozoon is clearly seen outside, separated from the entrance cone only by the thickness of the vitelline membrane with which it is in contact.

Fifteen minutes after insemination essentially the same condition persists. The entrance cone is homogeneous, shading off into the surrounding yolk-filled protoplasm. It stains very dark in iron haematoxylin. The head of the spermatozoon appears exactly as before.

Twenty minutes after insemination the entrance cone has flattened out, but the spermatozoon is still external to the membrane. The substance of the entrance cone is, however, as readily recognized as when it projected above the surface of the egg.

About thirty-seven minutes after insemination (metaphase of first maturation division) the sperm is still readily found on the exterior of the vitelline membrane external to the substance of the entrance cone which is now lens shaped. The substance of the entrance cone is homogeneous and it stains less than before; it is sharply marked off from the unaltered egg cytoplasm by a layer of small basophile granules. In the center of its external face is a sharply differentiated granule which stains intensely black in iron haematoxylin, and which is connected to the sperm head by a fine thread passing through the vitelline membrane; penetration has already begun.

Forty-three minutes after insemination (late metaphase of the first maturation division) the entrance cone sinks into the egg-cytoplasm, and the head of the spermatozoon begins to be drawn within the egg in the form of a thick thread, less than one-third the diameter of the sperm head, however. The sperm nucleus is being drawn through the small perforation in the vitelline membrane.

Forty-eight minutes after insemination (stages of anaphase of the first maturation division), nearly all of the sperm head is drawn into the egg in the form of a thick thread several times longer than the original sperm head. Before the head is entirely within the egg its inner end begins to swell and becomes vesicular. The entire entrance cone penetrates the egg-protoplasm always retaining its original connection with the apex of the spermatozoon, so that the original orientation of the sperm is preserved and may be readily recognized after penetration.

Fifty-four minutes after insemination (telophase of the first maturation division), the entire head of the spermatozoon is within the egg. The tail and middle piece usually remain without.

As I intend to publish a separate account of the interesting details of penetration of the spermatozoon, and as the later stages do not concern the present problem, I shall simply say, therefore, that as the united sperm-head and entrance cone penetrate farther into the egg cytoplasm, they rotate in such a way that the entrance cone which was originally in advance of the sperm nucleus comes to lie behind it. During the rotation the sperm

aster arises from the pole of the sperm nucleus opposite the entrance cone, thus in the position of the original middle piece.

Morgan has recently ('10), with entire justice as it appears to me, taken a stand against the current view that penetration of the sperm is due to mechanical boring into the egg. He believes that the presence of the sperm calls forth a reaction on the part of the egg that leads to the absorption of the former. There can be no question that the latter conception fits the case of *Nereis* much better than the former. In the first place the spermatozoon is absolutely motionless after its attachment to the membrane; in the next place the formation of the entrance cone shows a very decided reaction on the part of the egg to the presence of the spermatozoon; in the third place the retraction of the spermatozoon into a depression of the membrane is due to the retraction of the entrance cone; and finally, as I shall show in a subsequent cytological study, the inclusion of the spermatozoon within the egg appears to be due to activity of the substance of the entrance cone, and not to active penetration by the spermatozoon. The spermatozoon does not penetrate the egg, it is drawn in or engulfed.

*b. The later history of the sperm nucleus.* The sperm amphiaster is visible in the preparations all through the period of the second maturation division (fig 4). After the formation of the second polar body the sperm-nucleus begins to enlarge and the amphiaster gradually wanes, but it may be recognized up to the time of contact of the germ nuclei. The centrosomes of the first cleavage spindle then begin to appear. Whether or not they are continuous with those of the sperm amphiaster is a question which I shall take up in the next study of this series. The cleavage asters rapidly become very large and conspicuous (figs. 5 and 6). During the growth of the germ nuclei a considerable number of large granules staining strongly in iron haematoxylin appear in their immediate vicinity.

The main point of these observations on the normal fertilization, both in the living eggs and also in section, is to demonstrate for elucidation of the experiments following: (1) That membrane formation precedes penetration of the spermatozoon by a long time. (2) That the spermatozoon does not penetrate

the vitelline membrane and enter the egg until at least forty to fifty minutes after insemination, although its attachment to the membrane takes place immediately. (3) That the presence of the sperm-nucleus is readily demonstrable in all stages after penetration.

*B. Removal of the spermatozoon after membrane formation*

In the summer of 1909 I was studying the effects of centrifuging on the egg of *Nereis* with the aim of getting more data on the problem of polarity and the theory of formative stuffs. It soon became apparent that the effects of centrifuging varied with the stage of development, and so several series of experiments were made in which the eggs were centrifuged at regular intervals from before fertilization up to the time of the first cleavage.

The effects of centrifuging may be divided into three categories: (1) A certain proportion of centrifuged eggs develop approximately normally, the percentage varying greatly with the stage of centrifuging. (2) A certain proportion of eggs, varying at different stages, segment more or less abnormally, sometimes extremely so (*e.g.* meroblastic), and produce embryos with more or less pronounced abnormalities. (3) At certain stages of centrifuging a variable proportion of eggs fails to carry out even the first cleavage. The investigation of the causes of such failure to segment revealed the fact that it was owing to the removal of the spermatozoon after membrane-formation. It is the evidence for this statement that is now under consideration.

The results with reference to failure to segment were, in general, as follows:

1. If unfertilized eggs were centrifuged and then fertilized, all segmented, and a large percentage tended to develop quite normally.

2. A disturbing factor comes in shortly after insemination, owing to the fact that when the jelly is first secreted by the eggs it is so viscid that the eggs stick together in the bottom of the centrifuge in a mass which cannot be separated into its constitu-

ent eggs. The extreme viscosity of the jelly gradually disappears, and after about twenty minutes from insemination, the eggs no longer mat together. It is therefore difficult to investigate the effects of centrifuging on the developmental capacity of the eggs during the first ten or fifteen minutes after insemination. However, when the viscid stage begins to pass away and eggs can be separated from the mass for examination, the majority are found to undergo segmentation, as many as 98 per

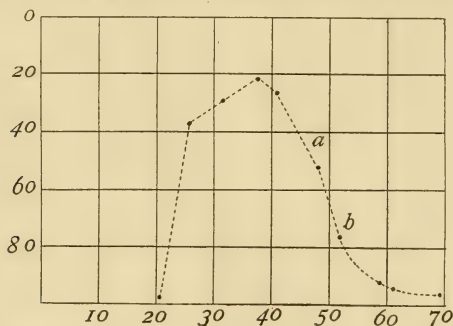


Fig. C. The effects of centrifuging on the power to segment in *Nereis*. The abscissae represent minutes from the time of insemination, the ordinates the percentage of eggs dividing. At position *a* penetration of the spermatozoon is just completed in most of the eggs. At position *b* the first polar body is extruded. Data from experiment 2, 1910.

cent in one case (experiment 2, 1910) twenty-one minutes after insemination.

3. For about the next thirty minutes (twenty-one to fifty-three minutes after insemination) centrifuging tends to inhibit the cleavage of a certain proportion of the eggs which gradually increases up to about thirty-seven minutes after insemination and then decreases again. For instance, in experiment 2 of 1910, of the eggs centrifuged twenty-one minutes after insemination 98 per cent segmented; twenty-six minutes after insemination 36 per cent segmented; thirty-two minutes, 33 per cent; thirty-seven minutes, 21 per cent segmented; forty-three minutes, 26 per cent segmented; forty-eight minutes, 52 per cent segmented; fifty-three minutes, 75 per cent segmented; fifty-

eight minutes, 90 per cent segmented; sixty-three minutes, 90+ per cent segmented; sixty-nine minutes, 95+ per cent segmented; control eggs, 99 per cent segmented. (See Fig. C.)

4. From this time on nearly all of the eggs segment after centrifuging until, during the anaphase and telophase of the first cleavage spindle, centrifuging again tends to inhibit cleavage.

The following table (Experiment 29) gives a fairly typical series of results. There were twelve such experimental series in all, more or less complete, giving concordant results except that in some at the period corresponding to 29D, 90 to 98 per cent were so affected that they failed to segment. On either side of this critical period there is a decreasing susceptibility to such injury by centrifuging.

## EXPERIMENT 29

*September 8, 1909*

DESIGNATION	TIME OF CENTRIFUGING AFTER INSEMINATION	PERCENTAGE SEGMENTED	REMARKS
29.....	Control (not centri- fuged)	100 per cent	
29A.....	Before insemination	100 per cent practi- cally	
29B.....	20 minutes	Majority	Eggs matted loosely
29C.....	30 minutes	Majority	
29D.....	41 minutes	20 to 30 per cent	
29E.....	51 minutes	70 to 80 per cent	
29F.....	66 minutes	90 to 95 per cent	
29G.....	79 minutes	100 per cent	
29H.....	95 minutes	100 per cent	
29I.....	114 minutes	100 per cent	
29J.....	121 minutes	Some unsegmented	
29K.....	127 minutes	Less than majority and these irregu- lar	Centrifuged during process of 1st cleavage
29L.....	149 minutes	Most segmented fur- ther	Centrifuged in 2-cell stage

Two major processes are going on in the egg at this time, viz.: maturation and fertilization. The injury is not primarily to the process of maturation, for the eggs that do not segment form the polar bodies; nor is it probable that there is a general

systemic injury to the egg protoplasm at this time not received at other times, when the fact that maturation continues and polarity is preserved in these eggs, is considered. It would, therefore, appear probable that the injury is to the process of fertilization itself, and this conjecture is completely confirmed by cytological study. The most conclusive experiment in this respect is no. 27, the details of which are as follows:

The eggs were fertilized at 9:28 A.M., September 4, 1909. Some of these were kept for control and all segmented normally. The remainder were centrifuged about 60 x 120 revolutions at a radius of 6 cm. in one minute, at the following times: 27A at 9:58 A.M.; 27B at 10:03; 27C at 10:08; 27D at 10:12; 27E at 10:16. About 20 per cent of 27A segmented, 5 to 10 per cent of 27B, 20 per cent of 27C, 50 to 60 per cent of 27D, and 75 to 90 per cent of 27E. Samples of the control and of each of 27A, 27B, 27C, 27D and 27E were preserved at 10:31 and 10:43 A.M.<sup>2</sup>

#### EXPERIMENT 27

*Eggs fertilized at 9:28 A.M., Sept. 4, 1909*

DESIGNATION	CENTRIFUGED	TIME AFTER INSEMINATION	SAMPLES PRESERVED	LIVING EGGS
27 Control.	Not centrifuged		(1) 10:31½ A.M. (2) 10:45 ½ A.M.	All segmented
27A . . .	60 x 120 rev. in 1 min. 9:58 A.M.	30 min.	(1) 10:30 A.M. (2) 10:43½ A.M.	About 20 per cent segmented
27B. . . .	60 x 120 rev. in 1 min. 10:03 A.M.	35 min.	(1) 10:30½ A.M. (2) 10:43½ A.M.	About 5-10 per cent segmented
27C. . . .	60 x 120 rev. in 1 min. 10:08 A.M.	40 min.	(1) 10:30½ A.M. (2) 10:44 A.M.	About 20 per cent segmented
27D. . . .	60 x 120 rev. in 1 min. 10:12 A.M.	44 min.	(1) 10:31 A.M. (2) 10:44½ A.M.	About 50-60 per cent segmented
27E. . . .	60 x 120 rev. in 1 min. 10:16 A.M.	48 min.	(1) 10:31 A.M. (2) 10:45 A.M.	About 75-90 per cent segmented

<sup>2</sup>Since the above was written this experiment has been repeated (Exp. 2, '10), with the added precaution of preserving a sample of the normal eggs corresponding to each stage of centrifuging, in order to make certain of the stages of fertilization in each case. The results completely confirm those of experiment 27, and the sperm was found to be external in the most critical stage (37 minutes after insemination in this experiment; see page 371).

Cytological study of the twelve lots of preserved eggs showed stages ranging from the metaphase of the second maturation spindle to the prophase of the first cleavage, the earlier stages of course being found in lot 1 in each case.

In the control lots it was easy to demonstrate the sperm nucleus at all stages to the formation of the first cleavage spindle. The sperm nucleus is rendered particularly conspicuous during the second maturation division by the large amphiasier that accompanies it (fig. 4), both lying in the yolk-free protoplasm. After the formation of the second polar body the sperm amphiasier gradually fades, but the sperm nucleus can be recognized by its position and by the remnants of radiations up to the time of union of the two germ nuclei; and in the later stages its presence may be inferred by the degree of development of the cleavage amphiasier and the number of chromosomes. There is, therefore, no time from the beginning of the second maturation division up to the formation of the first cleavage spindle when the presence of the sperm nucleus cannot be readily demonstrated.

In the study of the serial sections of the control eggs I found no egg in which, all sections being present, the sperm nucleus could not be demonstrated. In the serial sections of 27A, the sperm nucleus could be recognized in only about 37 per cent of the eggs; in 27B in only 10 per cent to 20 per cent; in 27C in about 25 per cent; in 27D in about 53 per cent; in 27E in about 76 per cent. The stages of maturation of lots A to E corresponded very closely with the stages of maturation of the control eggs killed at the same time.

It is a relatively simple matter to demonstrate the presence of the sperm nucleus, for a single positive observation suffices; but, to be sure of the absence of a sperm nucleus from any particular egg, it is necessary to examine practically every section of the egg, and the absence of two consecutive sections is sufficient reason for excluding an egg from the count. This may be one reason why the number of eggs in the different lots shown to contain sperm nuclei tends to be somewhat larger than the estimate of the number of eggs that segmented. Another reason

probably is that a sperm nucleus may persist to a certain extent even if injured and unable to produce the full fertilizing effect and cause cleavage.

A third reason for discrepancy in the results is that abnormalities of maturation may be produced by centrifuging which render the determination of the sperm nucleus more difficult than usual. It frequently happens that, as the first maturation spindle is driven from the surface by the centrifugal force, it divides before it reaches the surface again, producing two maturation nuclei within the egg. The two second maturation spindles may then unite to form a tetraster, one pole of which approaches the surface and a single polar body is formed, leaving three nuclei within the eggs (fig. 7). Such eggs were readily recognized by the absence of the first polar globule, and by the presence of the extra nuclei. But it was sometimes difficult to determine in certain stages whether there were only three nuclei, the sperm nucleus being absent, or four, the sperm nucleus being present.

A fourth reason for a certain discrepancy between the estimate of the number of eggs that segmented and the number determined to have sperm nuclei might be that at the time the experiment was made the importance of *exact* determination of the number of eggs that segmented was not realized, and the determination was made only roughly. Putting the results side by side we have:

	PERCENTAGE OF EGGS OBSERVED TO DIVIDE IN LIVING CONDITION	PERCENTAGE DETERMINED BY SERIAL SECTIONS TO POSSESS SPERM NUCLEI
27 Control.....	All	All
27A.....	About 20 per cent	About 37 per cent
27B.....	5 to 10 per cent	About 10-20 per cent
27C.....	About 20 per cent	About 25 per cent
27D.....	About 50-60 per cent	About 53 per cent
27E.....	About 75-90 per cent	About 76 per cent

Considering the various sources of error, the agreement is very close except in 27A. But in this case we do not have to

explain why eggs in which the sperm nucleus was absent segmented but on the contrary, why certain eggs that possessed the sperm nucleus failed to segment, which is a very different thing. There is no evidence that any egg in which the sperm nucleus was absent succeeded in dividing.

The general conclusion that removal of the spermatozoon at the times noted in the experiments involves incomplete fertilization, is sufficiently demonstrated by the results.

Let us call the stage at which the spermatozoon is eliminated in the greatest proportion of eggs, the critical period. The exact number of minutes from the time of mixing eggs and sperm to this stage varies of course through the season, owing to the variations of temperature. Moreover, it is not *exactly* determined in all experiments, for in some the stages of centrifuging fall on either side of it. This being understood, we may note that in eight experiments the critical period occurred at from 25 minutes to 40 minutes after fertilization. This is quite a wide variation, but when the time is represented as a fraction of the entire period between fertilization and the first cleavage, it is found that in all cases the period up to the critical period is between 27 and 33 per cent of the total time up to the first cleavage. It is obviously a corresponding stage in all cases, for the observed differences fall within the chances of error, viz: that the critical period is hit exactly in only very few experiments, and that the time of beginning of the first cleavage must be stated rather arbitrarily on account of the variation in rate of individual eggs.

The critical period occurs shortly before the penetration of the spermatozoon into the egg. We noted in the first part of this paper that the penetration of the spermatozoon is extremely gradual; my observations on this point, both from the study of the living egg and also of sections, show that it requires forty to fifty minutes for the head of the spermatozoon to disappear through the membrane.

As the most critical period comes in the great majority of experiments from thirty-five to forty minutes after insemination, it is obvious that the spermatozoon is in some way prevented

from entering the egg. The explanation is comparatively simple; the spermatozoon is imbedded in the jelly by which the egg is surrounded. When the jelly is first formed it is very viscid, and adheres to the eggs during centrifuging so that they mat together in the centrifuge. However, this stage passes and the result of centrifuging is then to separate the jelly from the eggs. In many cases the jelly carries off the attached spermatozoon with it. After penetration this can of course no longer happen. The curve of variation of the per cent of eggs centrifuged before penetration that fail to segment is due to the following factors: (a) At about twenty-five minutes the sperm head is sunk in a deep depression of the membrane and hence is less likely to be torn away by the jelly; (b) the change in consistency of the jelly presumably extends from without inwards; hence at first the innermost layer in which the spermatozoon is imbedded tends for a time (also presumably) to remain with the egg; (c) the time of penetration varies somewhat in any lot of eggs. These facts together would explain why the percentage of eggs that fail to segment after centrifuging rises to a maximum and sinks again to a minimum in the successive stages of centrifuging.

The fact that centrifuging inhibits cleavage in a small per cent of the eggs from fifty to fifty-five minutes after insemination, leads me to suspect that in some cases the sperm may be destroyed after its penetration into the egg. In experiment 2, 1910, for instance, cleavage was inhibited in 25 per cent of the eggs centrifuged fifty-three minutes after fertilization; in the control eggs killed at the time of centrifuging, penetration of the sperm is completed in the great majority of eggs, though it is found external still in a very few; it is difficult to estimate the per cent of the latter, but the impression is that it is less than 25 per cent. However, it is impossible to confirm this, and I mention the matter here to call attention to the error in my first paper read before the Central Branch of the American Zoological Society (Abstract in Science, vol. 18, p. 36, May, 1910), in which I stated that the destruction of the sperm nucleus followed penetration. A renewed study of the penetration has proved that this is not the case, usually at least.

We are not, of course, free to infer that fertilization is complete as the stimulus to development immediately after penetration of the spermatozoon. The experiments prove directly that in the egg of *Nereis* the stimulus of the spermatozoon as the impulse to development consists of two distinct parts: (1) an external stimulus that causes membrane formation and which is sufficient of itself to induce the maturation; (2) an internal stimulus dependent on penetration of the spermatozoon. How long after penetration fertilization is still incomplete cannot be decided on the basis of these experiments.

In concluding this section, we may note that the cause of failure to segment following centrifuging during the anaphase of the first cleavage is an entirely different one. The cause in this case is the breaking up of the karyokinetic figure and function, and dispersing the chromosomes. This is readily demonstrated in sections. In the case of eggs centrifuged at the 'critical stage', the sections show that the maturation spindle receives no injury from centrifuging, but appears coherent and normal in the sections. The sections of eggs centrifuged during the anaphases of the first cleavage show the chromosomes dispersed through the cytoplasm and the cleavage spindle no longer coherent, but broken up. In the first case the cause of failure to segment is elimination of the sperm-nucleus, as shown by study of series 27. This cannot be the cause in the second case, and a sufficient explanation of the failure to segment is found in the destruction of the karyokinetic figure.

*C. Comparison of completely and partially fertilized eggs in later stages*

We have noted so far that definite proportions of eggs centrifuged at definite periods in the process of fertilization fail to develop a sperm-nucleus, and that similar proportions of the same lots of eggs when left to develop fail to undergo segmentation. The facts (1) that all the control eggs of the same lot segment, and (2) that the centrifuged eggs that fail to segment, nevertheless had formed the fertilization membrane and under-

gone maturation, prove that the unsegmented eggs had received at least the first stimulus of fertilization. It was also shown that the critical period for suppressing segmentation by centrifuging occurs at a time shortly before entrance of the spermatozoon, and that it is due to prevention of penetration. The partially fertilized eggs, therefore, resemble the normal ones in the fact that membrane formation and the first stimulus to development are called forth by action of the spermatozoon, and they differ from the normally fertilized eggs in that the internal egg protoplasm has not received the direct stimulus of the spermatozoon. A cytological examination of such eggs could not fail to be of interest and might give some clue to the internal function of the spermatozoon in fertilization.

Both polar bodies form regularly in such eggs as already noted, and the egg-nucleus (female pronucleus) arises and attains the same size as in normally fertilized eggs. The chromosomes of the first cleavage spindle then form in the usual fashion and at the usual time, accompanied by disappearance of the nuclear membrane. But, whereas, in the presence of a sperm nucleus, cytoplasmic asters accompany these processes and a spindle rapidly arises during the prophases of the first cleavage, in the absence of the sperm nucleus there is absolutely no sign of cytasters or evidence of spindle formation. The chromosomes lie naked in the cytoplasm surrounded by a clear area (fig. 7).

Each chromosome then splits longitudinally in the usual fashion, but the halves do not separate. At the time of the telophase of the normal first cleavage there is a tendency to scattering and breaking up of the chromosomes. When the normal eggs have reached the two and four-celled stages, the scattering and breaking up of the chromosomes have progressed much farther in the unsegmented eggs, and in the course of two or three hours there remains no differentiated nucleus or chromosomes, but only numerous chromatic granules scattered throughout the cytoplasm.

The behavior of the partially fertilized eggs may be compared on the one hand with that of normally fertilized eggs and on the other with that of eggs caused to mature by centrifuging. As com-

pared with the former, the chief difference observable by cytological methods is the entire absence of the achromatic part of the karyokinetic figure. The differences in later stages may be conceived as secondary effects of this defect or of the conditions determining such defect. When eggs are caused to mature by centrifuging the process begins as in normally fertilized eggs by the breaking down of the cortical layer and formation of the jelly; the germinal vesicle ruptures and the two maturation divisions follow. After the completion of the maturation the chromosomal vesicles of the egg nucleus usually fail to unite perfectly, and in a little while they separate and scatter in the cytoplasm without formation of chromosomes, so that each egg appears to possess a considerable number of small nuclei. In a few cases the first indications of chromosome formation may be observed in the vesicles shortly after maturation but not later. Subsequently the chromosomal vesicles appear to dissolve in the cytoplasm liberating small chromatic nucleoli.

The partial stimulus of the spermatozoon is thus somewhat more effective than the mechanical shock of centrifuging, though both produced the same initial changes, apparently equally well. This may possibly be due to entrance of a small amount of matter from the spermatozoon; for at the critical period the perforatorium of the sperm has penetrated the membrane and is imbedded in the entrance cone.

#### *D. General discussion*

The general conclusion that the function of the spermatozoon in the stimulus to development involves at least two factors has already been clearly stated by Boveri ('07) and Loeb ('09b): According to Loeb, one factor is the cytolysis of the "very thin cortical layer of egg"; but while this stimulates development, the latter is often abnormal and therefore usually comes to a halt. A second process is necessary to ensure more normal and lasting development (Loeb '09b). Apparently Loeb is not very clear concerning the nature of the second factor, but is inclined to regard it as inhibiting the cytolysis which he conceives to be

begun as the first factor of the developmental stimulus. This conclusion was formed as a result of two kinds of experiments: In the first Loeb found that the best results in artificial parthenogenesis are obtained, in the egg of a Californian sea-urchin, by a double treatment: first using a cytolytic agent and then following it by treatment with hypertonic sea-water, or by inhibiting oxidation for a while. In the second class of experiments Loeb and Elder found that mere membrane formation might be induced in sea-urchin eggs by external contact of starfish spermatozoa, but farther development did not take place except in the relatively few cases in which the spermatozoon entered the egg (Loeb '09b, p. 249), or unless the eggs were treated after membrane formation with hypertonic sea-water. Although this experiment is complicated by the hybridizing, yet it demonstrates the same distinction between external and internal functions of the spermatozoon in fertilization that I have shown for *Nereis* by a different method.

Artificial parthenogenesis may be induced in the sea-urchin egg without membrane formation and this fact appears to me to indicate that the internal function of the spermatozoon is probably at least as fundamental as the external function (membrane formation), though, as Loeb points out, development without membrane formation takes place in a less normal fashion than after membrane formation. But inasmuch as we may have membrane-formation without development, and development without membrane formation, it would seem premature at least to consider membrane formation as the chief function of the spermatozoon in fertilization.

The experiments described in this paper show that in *Nereis* fertilization by the spermatozoon is incomplete after the formation of the membrane. The question then arises, when is the function of the spermatozoon in fertilization completed? Ziegler's and Wilson's experiments show that it is incomplete even some time after entrance of the spermatozoon. Ziegler's experiments ('98) consisted "in so constricting the egg of the sea-urchin after penetration of the spermatozoon that the one part contains the sperm nucleus, the other part the female sex-nucleus. The

part that contains the sperm nucleus undergoes cleavage and develops farther; in the other part the female sex-nucleus undergoes remarkable transformations, dissolving and reappearing, a process which is repeated several times." In spite, therefore, of the presence of the sperm-nucleus in a constricted part of the same egg, the part containing the egg nucleus was not fully fertilized. It made abortive attempts at division, but the karyokinetic figure was too feeble to carry the process through.

Wilson observed ('03) that if the fertilized eggs of *Cerebratulus* be cut in two shortly after the penetration of the spermatozoon "only a single fragment develops even though the fragments be refertilized immediately after the operation. In such cases it is almost invariably the nucleated fragments that develop, but in a very few cases I have observed that the enucleated fragment develops, while the nucleated one forms the polar bodies, but proceeds no further." By the nucleated fragment in this case Wilson means the fragment containing the maturation spindle. Farther on he adds "The few cases in which the enucleated fragment of the bisected fertilized egg develops are doubtless those in which the plane of section separates the sperm-nucleus from the egg-nucleus." This is indeed the only possible explanation. In such cases the fragment containing the egg-nucleus is only partially fertilized.

Boveri has also observed that if freshly fertilized sea-urchin eggs be broken into fragments by shaking, certain of the fragments contain the egg nucleus alone. If such fragments are not subsequently entered by a spermatozoon, the nucleus enlarges, dissolves and reappears again; but they do not segment ('96). Later he observed that such pieces may divide at least twice ('02).

These experiments demonstrate that fertilization is still partial even some time after entrance of the spermatozoon into the egg; but they do not show at what stage it is complete. Boveri's very interesting observations on 'partial fertilization' in the sea-urchin egg ('88 and '90) carry the solution of the problem a step farther (cf. also Teichmann '02). In the experiments which furnished the material for his observations both eggs and sperm were weakened, the former by standing for fourteen hours

in sea-water and the latter by treatment with KOH prolonged to a stage in which only a small percentage of the spermatozoa continued to move. Under these conditions in a large number of eggs the sperm aster separated from the sperm nucleus, which was usually left on one side, and proceeded alone to conjugate with the egg-nucleus. Thereupon the cleavage spindle formed with the egg-nucleus alone, and segmentation of the egg ensued. In the four-cell stage usually, but sometimes in the two or eight-cell stage, the sperm nucleus united with one of the segmentation nuclei. Boveri concludes from this and other results that the fertilizing action of the spermatozoon consists in the introduction of a centrosome into the egg.<sup>3</sup> When this has united with the egg nucleus, with or without participation of the sperm nucleus, fertilization would be complete; or with the sperm nucleus alone in merogony it likewise completes fertilization.

Boveri has used the term 'partial fertilization' for the phenomenon just described, although he admits that it is a misnomer. It is unfortunate that such a significance should have come to be attached to the expression, because, as has been shown, my own results and those of Ziegler, Wilson and Boveri himself prove that partial fertilization in the literal sense really occurs. The various stages of partial fertilization as shown by the results in the literature on the subject are:

1. External contact alone by the spermatozoon producing,
  - a. Formation of the fertilization membrane, (Loeb and Elder for sea-urchins, Lillie for Nereis).
  - b. Maturation and formation of the chromosomes from the egg nucleus without spindle, (Lillie for Nereis).
  - c. Maturation and cleavage to stereoblastula, (Bataillon: hybrid union of eggs of *Pelodytes punctatus* and *Bufo calamita* with sperm of *Triton alpestris*).
2. If the spermatozoon be removed shortly after entrance,
  - a. Maturation alone may result, (Wilson on *Cerebratulus*).

<sup>3</sup>Herbst ('07 p. 202 and '09 p. 277) interprets Boveri's 'partial fertilization' as a combination of parthenogenesis and fertilization. Such an interpretation does not, however, explain Boveri's account of the behavior of the sperm-centrosomes.

b. An abortive karyokinetic figure may form with the egg nucleus alone, (Ziegler and Boveri on sea-urchins).

c. In some cases at least two cleavages may result, (Boveri on sea-urchins). Boveri's so-called 'partial fertilization, is really full fertilization, so it does not come in this series.

The difference in reaction of the egg-cytoplasm to its own nucleus and to the introduced part of the spermatozoon can be explained on only one of two grounds; either in the general sense of Boveri's theory on purely morphological grounds, or on the ground of a chemical difference, presumably of sexual origin, between the egg on the one hand and the sperm on the other. The latter form of interpretation seems to me preferable because it is a physiological interpretation which takes cognizance of the sexual factor in fertilization.

Boveri's theory of fertilization rests on the identification of the centrosomes of the sperm aster with a definite formed element (centrosome) introduced into the egg by the spermatozoon; but it has never been demonstrated in any case in all the literature on the subject of fertilization that the centrosomes of the cleavage spindle, or indeed of the sperm aster, are derived from any definite formed element of the spermatozoon. Boveri himself admits this in his sixth cell study ('07, page 266); and so long as definite proof of the continuity of the so-called sperm-centrosomes from the spermatid up to the formation of the first cleavage spindle is lacking, all of Boveri's observations are open to another interpretation than the one he has given; to the interpretation, namely, that the sperm asters represent a reaction of the egg cytoplasm to a male element, or at least a foreign element, represented for the most part by the sperm nucleus.

The biological analysis of fertilization seems to me to rest now upon the problem of the origin of the sperm aster in the egg. More crucial evidence is needed on this point, and I do not believe that any refinement of cytological technique will give the result. Experimental evidence is needed; either, as Boveri ('88) suggested, the introduction of a non-nucleated spermatozoon in the egg to prove whether or not the sperm asters would arise without the nucleus and fertilize the egg, or the introduction of only the

anterior part of the sperm into the egg to prove whether or not the sperm nucleus without the centrosome, which is contained in the middle piece of the spermatozoon, would cause the production of asters in the egg-cytoplasm.

As Boveri, among others, has pointed out, there is not only one, but several stages of inhibition in the history of the egg. This may be illustrated by noting the stages at which in the eggs of various animals the need for fertilization arises. In some eggs it is before the rupture of the germinal vesicle (*e.g.*, *Nereis*), in others at the time of the mesophase of the first maturation spindle (*e.g.*, *Chaetopterus* and *Cerebratulus*), in others again after the formation of the first polar body (*e.g.*, *Amphioxus*, amphibians), in others again, not until after the formation of both polar bodies (*e.g.*, sea-urchin). There is no doubt that the last stage of inhibition is the most difficult one to overcome, both because many eggs pass by the earlier stages without apparent specific stimuli and also because it is possible to cause eggs that normally stop at the first or second stage of inhibition to pass on to the last stage by stimuli that are ineffective when this stage is reached, (*e.g.*, *Nereis* as noted in this paper and *Chaetopterus* as noted in various earlier papers).

The nature of the inhibition that causes the need for fertilization is a most fundamental problem. Is it the same in all these cases, *i.e.*, a gradually increasing inhibition that may be effective before maturation, but in some cases not until maturation has progressed a certain distance, or even until it is complete?<sup>4</sup> If it is the same cause at all these stages then it is certain that the need for fertilization is not due to any defect of the egg-centrosomes, for the pause takes place in *Chaetopterus* (*e.g.*) while the egg-centrosomes are at the very height of their activity. If,

<sup>4</sup>Bataillon ('10) holds the view that the nature of the inhibition is the same whether the arrest is at the stage of the resting nucleus or in the height of karyokinesis. He holds the view that the inhibition is due to accumulation of excretory products and that the stimulus to development is essentially a process of elimination. Bataillon's paper was received after my own was completely written. His interesting results will be considered more fully in my next paper. In Part I of the present paper (last paragraph) I have presented a view similar in some respects to Bataillon's.

therefore, we are to hold to the theory of Boveri in its literal sense, we must believe that there are different kinds of inhibition. However, it is, I believe, simpler and more logical to hold that the inhibition differs only in intensity at these various stages; and this point of view seems to be supported by the fact that the same stimulus which at a lower intensity will cause only maturation to take place in *Chaetopterus*, at a higher intensity will cause differentiation also to proceed, though in this case without cleavage. Boveri ('07), however, holds that there are different kinds of inhibition, that the postulated degeneracy of the egg-centrosomes after maturation is in a sense the more primitive, and that other kinds have been secondarily acquired, a point of view that gives a more or less definitely teleological aspect to the question.

From a physiological point of view we might inquire, what are the conditions that cause the postulated sudden degeneration of the egg-centrosomes? Such a condition if found, would be nearer the fundamental cause of inhibition of the egg and it might turn out to be the same cause that conditions in so many cases an earlier arrest of activities in the egg.

The experiments on artificial parthenogenesis are sometimes regarded as involving the entire problem of fertilization. But if it be true, as many believe, that biological fertilization, (if I may be pardoned such an expression) is fundamentally a sexual reaction, then the physico-chemical analysis of fertilization must compass the entire problem of sex, which is much wider than the problem of parthenogenesis. The physico-chemical analysis of fertilization has dealt, up to the present exclusively, with the latter problem, and for this reason the earlier title of such studies 'artificial parthenogenesis', seems to me much more fitting than 'chemical fertilization' which is sometimes loosely used. From the zoological point of view, at least, parthenogenesis and fertilization are not interchangeable functions. There is a factor present in fertilization which is absent in parthenogenesis, and the latter is never the exclusive mode of reproduction among animals. The biological analysis of fertilization therefore involves problems that do not occur in the physico-chemical analysis of parthenogenesis.

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## PLATE I

### EXPLANATION OF FIGURES

1 Axial section of an unfertilized normal ovocyte of *Nereis*, fixed in Flemming's fluid, weaker solution. In this fixing fluid the yolk granules swell and tend to run together. The oil drops are dissolved out in the preparation and are represented by empty spaces. *v.m.*, vitelline membrane. *c.l.*, cortical layer, from which the jelly is formed.

2 Section of an egg of *Nereis*, fixed in Meves' fluid five minutes after insemination. The cortical layer is already somewhat reduced in thickness. The yolk granules are not swollen. The oil drops are not dissolved out. The section is approximately horizontal. *c.l.*, remains of cortical layer; *v.m.*, vitelline membrane.

3 Section of an egg of *Nereis*, fixed in Meves' fluid fifteen minutes after insemination. The cortical layer has entirely disappeared, and the perivitelline space is formed. The germinal vesicle is breaking down and the first maturation spindle is forming. *p.v.*, perivitelline space. *v.m.*, vitelline membrane.

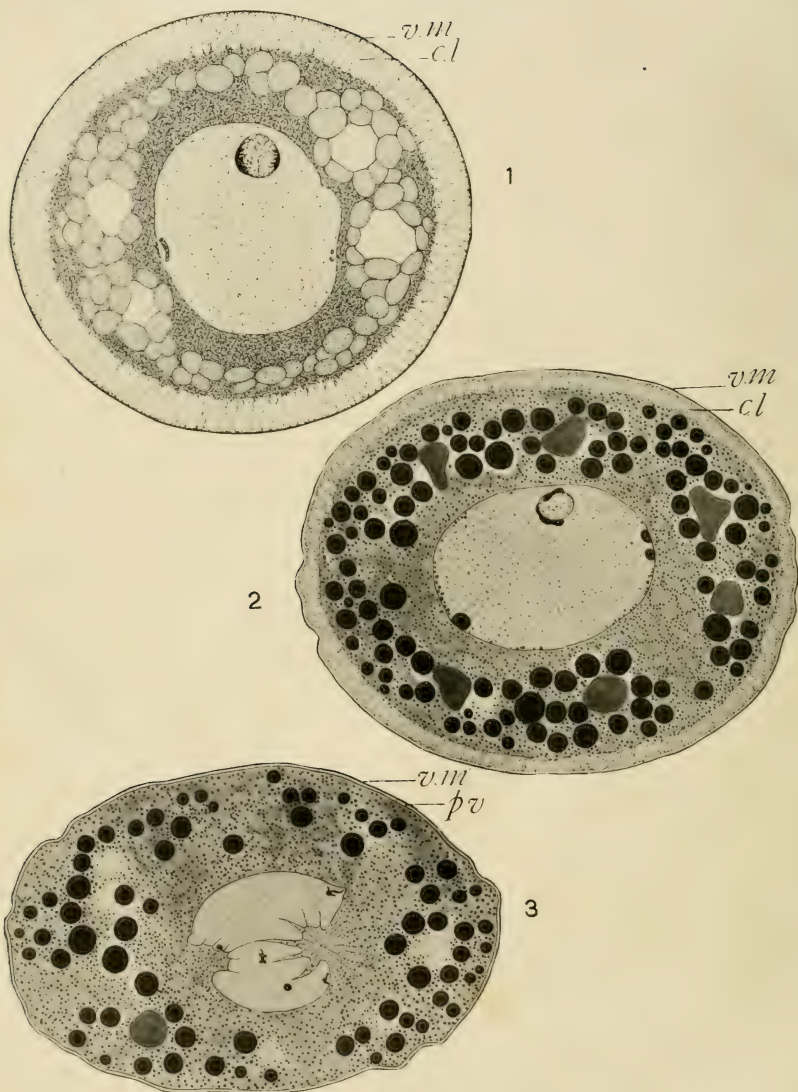
4 Section of an egg of *Nereis*, fixed in Meves' fluid fifty-seven minutes after insemination. Only one centrosome of the sperm amphiaster is shown.

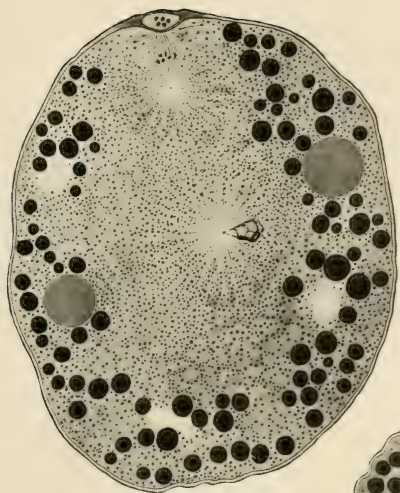
5 Section through the first cleavage-spindle of *Nereis*, normal, one hour and twenty-seven minutes after insemination.

6 Tetrapolar second maturation spindle of *Nereis*. See text for description (p. 378). Three egg nuclei are formed in such a case.

7 Egg nucleus of egg of *Nereis* in which the spermatozoon was removed by centrifuging. The chromosomes of the first cleavage are formed, but there are no asters. Cf. fig. 5.

All figures drawn with the camera lucida with Zeiss comp. oc. 6 and 2 mm. hom. oil im. obj.

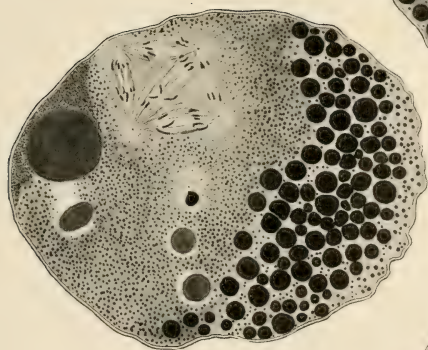




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# THE GROWTH AND DIFFERENTIATION OF THE CHAIN OF CYCLOSALPA AFFINIS CHAMISO

WM. E. RITTER AND MYRTLE E. JOHNSON

*From the Laboratory of the Marine Biological Association of San Diego*

TWENTY-FIVE FIGURES

FOUR PLATES

## CONTENTS

Purpose of the research.....	396
1. Special.....	396
2. General.....	396
Brief description of the species.....	398
Measurements of the zooids of the wheels and of a portion of the chain not yet transformed into wheels.....	399
Treatment of the quantitative data.....	406
Attempt to connect the formation of wheels with morphological, physiological, and mechanical phenomena presented by the animals.....	414
1. Segmentation of the stolon, and the deploying point.....	414
2. Position and relation of the zooids in the chain from the deploying point to the twist.....	414
a Shifting of the zooids.....	414
b Peduncles and foot-pieces.....	417
c Emergence of the chain to the outside world.....	417
d Twist in the chain.....	418
e Reduction of the foot-pieces, first break in the chain, and formation of the first wheel.....	418
3. Comparison of the rate of growth of the chain as a whole with the rate of other animals.....	419
Discussion of the observations from the causal standpoint.....	420
1. Cause of the twist.....	420
2. Unequal growth of zooids and foot-pieces as a factor in the breaking up of the chain.....	422
3. Impossibility that the character of the blood supply to the zooids can be the cause of the size scheme within the wheels.....	427
4. Unlikelihood that the wheel arrangement of the zooids in <i>Cyclosalpa</i> has, as believed by Brooks, anything to do with the position of the first four blastozooids of <i>Pyrosoma</i> .....	429

The larger significance of such studies.....	431
1. Supplementing biological with quantitative observations .....	431
2. Natural periodicity in organisms and exacter methods in biological research.....	432
3. The inadequacy of treating periodicity, generally, as an aspect of fluctuating variation .....	440
Bibliography .....	444

## PURPOSES OF THE RESEARCH

### 1. *Special*

One of us (Johnson, '10) has shown that the individuals of the blocks into which the chains of blastozooids of *Salpa fusiformis-runcinata*, *S. cylindrica*, and *S. zonaria-cordiformis* become differentiated, fall into size schemes, or systems.

The question naturally arises, how general is this phenomenon among salpae? The possibility that the wheel grouping in *Cyclosalpa* corresponds to the block grouping in *Salpa* proper, occurs to one rather readily in spite of the conspicuous differences between the two. If this conjecture be right, we should expect to find a size scheme of zooids in the wheels of *Cyclosalpa* similar to that in the blocks of *Salpa*. That such a scheme exists in the wheels even more pronouncedly than in the blocks, the sequel will show.

### 2. *General*

This much more evidence is consequently adduced favorable to the idea of correspondence between the wheels and the blocks. But what do we mean by correspondence? In a general sense the blocks and wheels undoubtedly correspond: both are groups of similar organisms similarly located with reference to the parent zooid. This much of correspondence is recognizable to cursory inspection. Does the discovery of a similar size scheme among the zooids in the groups in the two species advance our interpretation of these organisms much if at all? Does it amount to anything more than a recognition of one more resemblance? According to the meaning that 'interpretation' and 'resemblance' have in most later biological writing, we must probably say no. We

must hold that unless the new correspondence includes somewhere what we hold as one or more 'causal factors' not much has been accomplished. If, for example, we extend the inquiry to the question of the dependence of the size scheme of the zooids upon growth and other internal factors on the one hand, and upon environmental factors on the other; and if here, too, we find further correspondence, our belief in the essential identity as we might say, unless standing for extreme exactness of expression, would be reached.

We shall see that the size scheme of the zooids in the wheels is almost certainly foreshadowed before the wheels themselves are. If this be so, then the resemblance between the *Cyclosalpa* chain and the *Salpa* chain is considerably closer before than after the wheels appear in the former. But it is difficult, if not impossible, to attribute the block production in the *Salpa* chain entirely to other than inherent factors, of which growth seems to be the most immediate. So far, therefore, as we can rely upon our evidence for the marking off of the *Cyclosalpa* chain into groups before the wheels are formed we seem to have placed the notion of correspondence between the wheels and the blocks on firm ground.

Evidence still more convincing perhaps, that the wheels and blocks correspond in a strict biological sense, in the sense that both are expressions of periodicity in growth, is found in the fact that growth and development are observed to be periodic in so wide a range of living beings. The growth of plants for example, appears to be nearly if not quite always of that nature. Finally, belief in the correspondence would, so far as we can see, reach high water mark, should it be finally made very probable that not only growth and development but all strictly biological processes whatever, are periodic. We are undoubtedly a long way from this last conception. Certain it is, though, that we now have sufficient facts to make the hypothesis of periodicity as warrantable as its opposite, namely, that certain phenomena are continuous in the sense of not being automatically interruptive and group-wise. We may now proceed to the handling of our data.

## BRIEF DESCRIPTION OF THE SPECIES

The species *Cyclosalpa affinis* (Chamisso) was taken in abundance at La Jolla from May to November, 1909, during which time most of the observational portion of this research was made. The longest chains and the largest wheels were brought in during the earlier part of this period, while the later catches yielded many specimens of the solitary form with short chains, and many medium sized single wheels.

Although the salpae do not survive for more than a day or two in the ordinary aquarium, the material has been sufficiently abundant to admit of considerable work, with the living specimens.

The two generations of this species differ markedly, as do those of all members of the genus. The intestine of the solitary form (fig. 11) is straight, extending nearly the full length of the animal, the anal opening being just back of the ganglion. The intestine of the aggregate generation, on the contrary, projects from the ventral side of the creature as a large, almost circular loop, (fig. 12) the anus being only a little to the left of the oesophageal mouth.

The solitary form has eight body muscle bands, according to our system of enumeration, while the aggregate generation has five on the dorsal and six on the ventral side. The hypophysis (*hyp.*), endostyle (*end.*), and gill (*gi.*) present much the same appearance in both forms. The orifices are also similar except that the solitary form possesses short, tail-like appendages one on each side of the atrial orifice. Our records show the maximum length of specimens of the solitary generation to be 15 cm. and of the aggregate 8 cm. In both generations the test is thin, soft, and highly transparent, without special thickenings. In the aggregate generation, projecting from the ventral side, is the broad, thin peduncle (*ped.*) by which the zooids are united to form the wheel, and within the pharyngeal cavity on the right side, two thirds of the way back, is the embryo. In the young solitary individual, the cleoblast, near the heart, and the remnant of the placenta, about one-third of the way back from the oral orifice, are both opaque, nearly spherical bodies and are very prominent.

The stolon originates just above the heart and extends straight forward along the median line. The zooids, as in other salpa chains, are first in single file, but at a certain point, the deploying point, shift to double file. The deploying point in this species occurs close to the anterior end of the heart, 3 or 4 mm. from the root of the stolon. At a point about two-thirds of the way between the heart and the branchial orifice, the chain bends downward and passes to the outside world through an opening in the test. Immediately outside the opening, the chain doubles back under the parent and turns over so that it appears to be greatly twisted at this point. Before the twist, the zooids are arranged in two nearly parallel rows along the common stolonie blood vessel. After the twist, they are arranged in wheels which are connected tangentially and contain six to sixteen zooids each. These wheels show a gradual increase in size toward the distal end (fig. 11).

MEASUREMENTS OF THE ZOOIDS, OF THE WHEELS AND OF A  
PORTION OF THE CHAIN NOT YET TRANSFORMED INTO  
WHEELS

Serial measurements were made of the zooids of a number of chains with and without wheels as well as of the zooids of separate wheels. Lengths only were taken.

The measurements of the wheels were made with dividers and the results are given in millimeters. Those of the unbroken chains were made with the micrometer eyepiece used in the Zeiss binocular microscope. A unit in the tables represents 0.1 mm. of actual length.

The zooids of Chain I were separated from the chain for measurement, but the others were measured while still on the chain. It appeared that the latter is the more accurate method, since separating the zooids, besides being a tedious process, is apt to distort and mutilate them. In these measurements, the posterior extremity was taken at the atrial orifice. The intestine was not included, as a slight difference in its inclination would make a difference in the apparent length.

In table 1 are given the lengths of the zooids of the unbroken portion of Chains I-VII. Table 2 gives the same data for the



41	16.5	16.8	13.5	13.3	14.4	14.3	6.7	6.3	5.3	5.0	11.9	11.8	15.1	15.0
42	16.9	17.2	14.2	14.0	14.7	14.6	6.8	6.5	5.2	5.1	12.7	12.4	15.6	15.0
43	17.0	17.2	14.5	14.0	14.9	14.8	7.0	6.7	5.2	5.2	12.7	12.7	16.2	16.4
44	17.5	17.0	14.8	14.0	14.8	15.4	7.2	6.7	5.3	5.3	13.4	13.1	16.8	16.7
45	18.0	18.1	15.9	13.9	15.7	15.9	7.5	6.8	5.3	5.3	13.7	14.1	17.8	17.8
46	18.0	18.1	15.9	13.9	15.7	15.6	7.7	7.0	5.4	5.4	14.1	14.3	18.3	18.3
47	19.0	19.0	16.7	15.5	16.3	16.3	7.9	7.1	5.4	5.4	14.8	14.3	18.8	18.8
48	20.0	20.1	17.2	16.9	17.2	16.6	8.1	7.1	5.5	5.5	15.2	15.2	19.3	19.3
49	20.0	21.0	17.9	17.0	18.1	17.3	8.2	7.4	5.5	5.5	15.6	15.5	20.3	19.9
50	20.8	21.0	17.9	17.0	18.5	17.9	8.4	7.5	5.9	5.6	16.2	16.1	20.0	20.3
51	21.8	21.8	19.0	18.2	18.8	17.9	8.7	7.9	6.0	5.6	16.7	16.9	20.4	21.6
52	22.0	22.3	20.7	20.0	19.2	17.8	8.7	8.0	6.1	5.7	17.1	17.4	20.4	20.6
53	22.7	23.0	21.0	20.1	20.5	18.3	8.9	8.2	6.1	5.6	18.1	18.0	21.0	21.7
54	23.3	23.8	21.5	20.7	20.7	18.9	9.0	8.3	6.2	5.6	19.0	19.0	22.3	23.4
55	24.3	24.8	22.0	21.2	21.3	19.5	9.3	8.5	6.2	5.8	20.3	19.9	23.3	24.0
56	26.7	27.0	22.8	22.0	22.0	19.9	9.3	8.8	6.4	5.8	21.2	20.6	24.3	24.8
57	27.5	27.0	23.5	22.0	22.8	20.8	9.5	9.1	6.4	5.8	21.4	21.2	25.5	25.8
58	27.5	27.0	23.3	23.0	23.0	21.4	9.7	9.3	6.6	6.0	22.4	22.0	26.0	27.0
59	27.5	27.5	23.6	23.0	23.0	21.9	10.0	9.5	6.7	6.2	23.2	22.7	27.0	27.8
60	29.0	29.0	24.8	23.5	24.0	23.0	10.4	9.8	6.9	6.5	23.5	23.5	28.0	28.5
61	29.8	30.1	25.3	24.0	24.1	23.2	10.9	10.3	7.1	6.7	25.0	24.5	29.2	31.2
62	30.5	30.0	26.3	25.0	26.3	24.5	10.9	10.8	7.3	6.8	25.5	25.0	30.4	31.2
63	31.0	29.0	26.5	25.5	27.8	25.1	11.2	10.8	7.4	6.9	26.8	26.3	30.8	33.3
64	32.5	31.0	27.0	26.5	28.0	26.1	11.2	11.1	7.5	7.2	27.3	26.8	33.3	33.0
65	33.0	29.0	28.3	27.4	28.9	27.0	12.3	11.7	7.7	7.3	28.6	28.1	34.5	34.5
66	33.7	33.0	28.8	28.7	29.6	28.5	12.5	11.8	8.0	7.4	29.6	29.7	35.8	34.5
67	35.0	33.0	29.7	29.8	30.8	28.7	12.9	12.1	8.4	7.7	31.0	31.4	37.5	36.5
68	35.0	37.0	32.0	31.5	31.3	29.2	13.4	12.5	8.4	7.8	30.8	31.0	39.2	37.0
69	36.5	37.5	32.5	32.3	32.6	29.3	13.7	12.9	8.6	7.9	32.0	32.0	39.8	39.0
70	37.0	38.0	33.0	32.3	33.1	30.3	13.9	13.3	8.7	8.0	33.0	34.0	41.5	40.0
71	37.0	40.0	33.5	33.0	35.0	32.7	13.7	13.6	8.7	8.0	33.5	34.8	42.0	40.0
72	37.5	34.5	35.0	34.3	35.0	32.0	13.9	13.3	8.9	8.5	35.8	35.0	45.1	41.0
73	38.0	40.0	35.5	34.5	34.8	33.8	14.6	13.7	8.9	8.5	36.0	36.3	43.3	42.8
74	38.5	42.5	37.0	36.5	36.3	34.6	14.9	13.8	9.4	8.8	37.0	37.0	43.8	43.8
75	39.0	42.0	38.5	37.0	36.0	36.5	15.5	14.7	9.6	9.0	37.0	35.5	44.5	44.5
76	41.0	45.0	40.3	39.8	36.3	36.0	15.5	14.9	9.8	9.1	37.5	38.8	46.8	46.8
77	41.0	45.0	42.0	42.0	36.3	36.0	16.9	15.7	10.0	9.6	37.5	37.0	50.8	50.8
78	43.5	47.5	44.0	41.0	36.3	37.0	16.9	16.0	10.3	9.8	39.0	39.0	53.0	53.0
79	46.0	51.0	45.1	42.0	37.0	39.9	17.9	16.8	10.6	10.4	42.0	42.0	53.0	53.0
80	48.0	50.0	46.8	41.3	39.3	40.2	18.1	17.0	10.8	10.5	42.8	42.8	55.8	51.8
81	47.0	53.0	48.5	46.5	41.5	41.6	18.1	17.6	11.0	10.7	44.0	43.5	52.8	53.8
82	50.0	54.0	50.5	48.0	43.8	42.5	18.0	18.2	11.3	10.8	45.2	45.3	57.2	57.2
83	50.0	55.0	60.0	49.0	45.0	44.7	18.4	19.3	11.9	11.3	48.0	47.8	64.5	64.5
84	55.0	55.0	60.0	50.5	46.3	45.0	19.9	20.0	12.1	11.5	51.5	46.7	65.8	58.5
85	53.0	58.5	53.0	52.8	48.2	47.6	19.7	20.5	12.1	11.5	52.4	48.7	67.0	63.3
86	55.0	60.0	53.5	54.0	48.2	47.6	20.9	21.3	12.6	12.1	54.8	51.0	69.3	66.2
87	55.0	62.0	56.8	57.0	48.1	48.5	21.9	20.9	12.9	12.5	56.8	53.0	74.2	71.8
88	50.0	61.5	57.0	59.0	49.8	50.0	22.4	21.8	13.0	13.1	56.8	54.3	75.5	72.5
89	61.0	60.0	60.0	60.5	50.0	50.5	20.8	22.6	13.0	12.9	56.8	54.3	74.2	71.8
90	61.0	61.0	60.0	64.5	49.0	50.5	20.8	22.6	13.0	12.9	56.8	54.3	74.2	71.8

TABLE 2

*Length measurements of the zooids of wheels of chains VII and VIII*

Horizontal lines indicate end of wheels.

Double lines indicate one wheel lost.

Unit—1 mm.

NO.	CHAIN VII		CHAIN VIII		CHAIN VIII—continued			CHAIN VIII—continued		
1.....	7.3	7.3	6.4	6.1	41.....	13.7	12.6	81.....	20.5	20.9
2.....	7.6	7.6	6.7	6.8	42.....	13.1	12.0	82.....	19.2	18.6
3.....	7.7	7.8	6.9	7.0	43.....	12.9	12.2	83.....	18.6	18.6
4.....	7.7	8.2	7.1	6.7	44.....	13.0	12.9	84.....	19.8	17.2
5.....	7.8	8.1	7.4	6.8	45.....	13.9	13.0	85.....	20.7	19.8
6.....	8.2	8.3	7.4	6.8	46.....	14.0	13.4	86.....	22.7	19.8
7.....	8.6	8.4	7.5	7.4	47.....	14.2	13.5	87.....	21.9	20.2
8.....	8.4	8.7	7.6	7.5	48.....	14.2	14.1	88.....	22.5	20.4
9.....	8.7	8.8	6.7	7.1	49.....	14.3	14.0	89.....	21.9	20.5
10.....	8.9	8.8	6.8	7.2	50.....	14.3	12.8	90.....	19.8	17.5
11.....	8.7	8.7	7.2	7.8	51.....	14.9	12.4	91.....	21.1	20.0
12.....	8.2	8.8	8.7	7.6	52.....	15.2	14.0	92.....	22.9	21.0
13.....	9.5	9.7	8.2	8.3	53.....	15.3	14.7	93.....	24.2	23.2
14.....	9.5	10.0	8.5	8.6	54.....	15.7	15.3	94.....	22.8	23.2
15.....	9.7	10.0	8.8	8.2	55.....	16.0	14.3	95.....	24.1	21.6
16.....	9.8	10.2	9.4	8.4	56.....	16.4	15.3	96.....	23.0	19.9
17.....	9.6	10.3	9.2	8.7	57.....	15.0	14.8	97.....	21.3	20.6
18.....	9.4	10.0	9.3	8.4	58.....	15.4	14.0	98.....	21.2	20.9
19.....	9.8	9.3	10.6	9.1	59.....	15.5	13.9	99.....	23.7	21.3
20.....	10.6	11.0	9.8	8.9	60.....	16.6	13.2	100.....	24.5	22.6
21.....	10.9	11.1	9.7	9.1	61.....	16.2	15.4	101.....	24.6	23.4
22.....	10.9	11.4	10.5	9.0	62.....	15.3	14.7	102.....	23.6	23.6
23.....	10.8	11.4	9.7	8.8	63.....	15.2	15.4	103.....	24.0	22.5
24.....	10.1	10.9	9.8	8.9	64.....	14.0	14.3	104.....	23.9	22.1
25.....	9.7	10.6	9.6	8.9	65.....	16.0	13.6	105.....	23.0	22.1
26.....	11.0	10.7	10.2	8.5	66.....	16.3	18.3	106.....	25.1	22.2
27.....	11.1	10.5	9.8	8.1	67.....	18.8	17.9	107.....	26.3	23.1
28.....	11.4	11.4	9.8	8.5	68.....	19.6	17.7	108.....	26.1	23.7
29.....	11.8	10.6	10.1	9.2	69.....	18.4	17.7	109.....	26.4	23.4
30.....	11.5	11.2	9.6	9.1	70.....	18.0	17.1	110.....	25.5	22.9
31.....	11.1	11.2	10.9	10.2	71.....	15.7	14.7	111.....	22.4	21.3
32.....	11.7	11.3	11.8	9.9	72.....	16.2	14.7			
33.....	11.1	11.4	12.1	10.5	73.....	18.8	17.3			
34.....	12.8	12.4	12.3	10.8	74.....	19.4	19.4			
35.....	13.0	11.8	12.3	10.7	75.....	19.7	19.4			
36.....	12.9	11.8	11.6	11.5	76.....	19.5	18.3			
37.....	12.2	11.7	12.0	11.5	77.....	18.6	18.4			
38.....	12.5		12.7	11.4	78.....	19.3	16.1			
39.....			13.4	11.4	79.....	20.5	19.1			
40.....			13.6	11.6	80.....	22.2	20.0			

wheel portions of Chains VII and VIII. In all, the unbroken portions of seven chains were measured. The number of zooids used depended upon the minimum size measurable. In four cases, 90 zooids were taken but in the other three chains only 80, 70 and 52 zooids respectively were large enough to be measured accurately. The measurements of all series are given but only two are plotted, the right-hand series of Chain II in fig. 1, and the right-hand

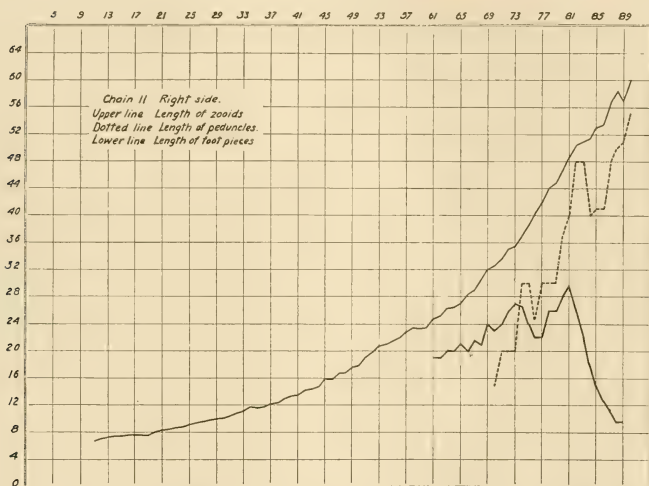


Fig. 1 Plot of length measurements of the zooids, peduncles, and foot-pieces of chain II, right series. Vertical distances represent length. Horizontal distances represent position in the chain.

series of Chain VII in fig. 2. In the latter figure, 1-90 are the zooids of the unbroken part of the chain while 91-108 are wheel zooids, the divisions between wheels being indicated by vertical dotted lines. Table 3 gives the measurements of zooids of several short chains of wheels which furnish figures for comparing graphs of wheels of various sizes. In each series of results here given except length of Chain I, two measurements were taken and these

TABLE 3

*Length measurements of zooids of various small groups of wheels.  
Unit—1mm.*

	GROUP A		GROUP B		GROUP C		GROUP D	
	FOUR WHEELS		FOUR WHEELS		FOUR WHEELS		TWO WHEELS	
	R.	L.	R.	L.	R.	L.	R.	L.
1.....	10.9	10.7	19.0	18.5	16.7	20.8	27.9	27.2
2.....	11.7	10.8	18.4	18.6	18.0	21.5	29.4	26.4
3.....	12.1	11.1	19.1	19.1	19.1	21.2	29.2	30.0
4.....	12.1	11.4	19.0	19.6	19.7	21.9	29.8	30.7
5.....	12.6	13.2	20.5	20.7	20.1	22.6	30.1	30.8
6.....	13.2	13.5	17.9	19.9	21.6	21.9	29.3	29.5
7.....	14.7	13.6	19.6	19.8	21.1	22.0	28.9	30.7
8.....	15.3	14.5	21.1	19.9	22.3	23.4	30.9	31.8
9.....	15.6	15.7	21.6	20.2	22.6	24.2	30.8	32.9
10.....	16.3	15.8	22.0	22.1	22.6	24.2	40.0	31.9
11.....	14.8	15.1	22.5	22.4	22.5	23.7	30.4	33.2
12.....	16.6	15.1	22.2	23.0	21.0	24.2	30.3	31.3
13.....	17.0	17.1	22.3	21.8	19.9	23.0		
14.....	16.9	16.7	23.3	21.8	22.3	24.0		
15.....	17.1	17.9	22.3	23.5	24.0	24.1		
16.....	18.5	18.5	24.6	24.2	25.5	25.0		
17.....	18.1	18.9	24.1	24.3	23.5	25.2		
18.....	12.7	15.9	23.0	23.1	25.0	25.7		
19.....	18.5	15.7	22.8	22.3	26.5	25.8		
20.....	19.4	18.8	24.6	24.0	27.6	24.0		
21.....	19.4	18.9	24.9	26.1	28.4	26.6		
22.....	18.9	18.6	25.8	25.4	27.8	27.3		
23.....	15.1	19.8	25.7	25.7	27.8	28.5		
24.....			24.9	25.7	23.8	28.4		
25.....				24.5		28.0		
26.....						25.9		

TABLE 3—CONTINUED

	GROUP E		GROUP F		GROUP G		GROUP H	
	THREE WHEELS		THREE WHEELS		TWO WHEELS		ONE WHEEL	
	R.	L.	R.	L.	R.	L.	R.	L.
1.....	23.4	23.0	22.3	22.9	21.3	21.0	25.4	23.1
2.....	24.6	24.8	23.8	23.3	21.9	22.2	25.1	24.5
3.....	25.2	25.7	24.4	23.9	21.6	22.6	25.0	25.1
4.....	24.7	26.1	24.8	23.9	22.3	24.2	24.9	25.1
5.....	25.1	26.5	25.0	24.7	22.4	22.6	23.9	24.8
6.....	23.6	25.3	26.3	25.1	21.3	23.2		24.4
7.....	24.9	24.1	24.2	25.0	22.0	25.2		
8.....	26.8	26.9	24.8	24.2	22.5	25.9		
9.....	27.6	27.2	27.2	25.9	24.3	26.8		
10.....	27.2	27.5	28.7	25.1	25.3	26.8		
11.....	27.7	27.7	28.6	26.0	25.7	26.1		
12.....	27.2	27.2	28.4	26.8				
13.....	27.4	27.5	28.4	27.9				
14.....	28.8	28.3	27.2	27.2				
15.....	28.7	30.0	26.6	27.2				
16.....	29.7	29.8	29.9	29.2				
17.....	29.2	29.2	29.8	29.8				
18.....	27.6	30.3	29.3	29.9				
19.....		28.2	30.4	30.3				

were averaged for the final result. Where the first and second measurement differed by more than 10 per cent, a third measurement was taken and the three figures averaged.

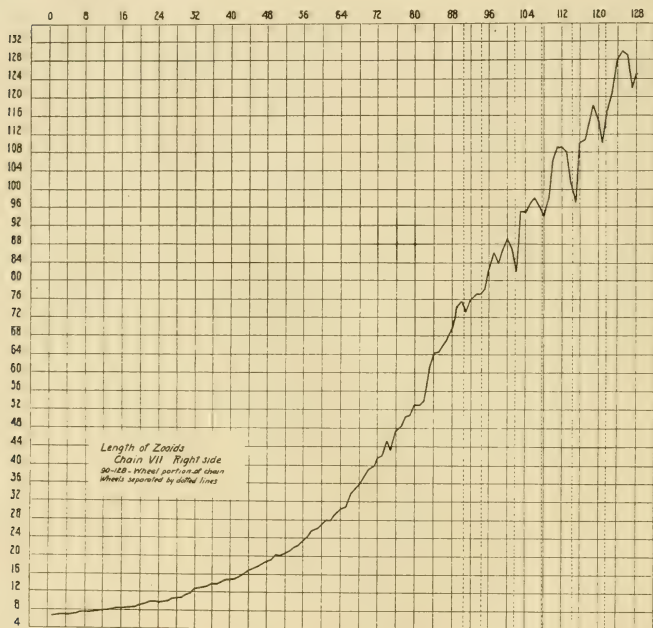


Fig. 2 Plot of the length measurements of the zooids of chain VII, right side, including wheels.

#### TREATMENT OF THE QUANTITATIVE DATA

At first glance, one sees a resemblance between the curves for the wheels of *Cyclosalpa affinis* and the blocks of *Salpa fusiformis-runcinata*. In both cases, the end zooids are smaller than those nearest them, the maximum values lying somewhere between, usually nearer the distal end.

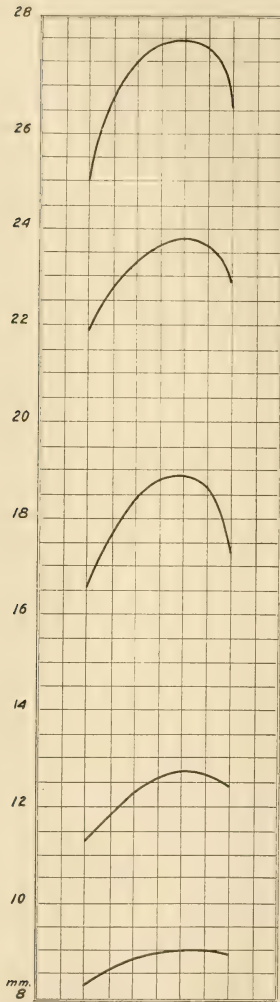


Fig. 3 Mean curves for wheels of various sizes. Vertical distances represent length of zooids. Horizontal distances represent position in the wheel.

Some variation in the graphs of wheels of different sizes was noted, and to make sure of its general trend, the data for all the wheels were considered. The wheels were first grouped according to size, Group A included wheels whose zooids averaged 5–10 mm. in length; Group B, 10–15 mm.; and so on. In Group A were ten wheels. Not only does the number of zooids in a wheel vary, but the number in one-half of a wheel is not always the same as in the other half. For this reason the ten wheels were regarded as twenty half wheels.

Among these twenty half wheels of Group A were three containing four zooids; one with five zooids; nine with six zooids; and seven with seven zooids. The corresponding values of the three four-zooid half wheels were averaged, the three first zooids together, the three second zooids, the three third, and the three last zooids. The result was a typical curve for a four-zooid half-wheel whose zooids have an average length of 6–10 mm. The five, six, and seven-zooid half-wheels were averaged in the same way. Similar computations were made for the other four groups and the results plotted. The graphs were smoothed and those for each size were averaged in order to get the typical curve for that size. These curves (fig. 3) show that the size *differences between the zooids of a half-wheel greatly increase as the zooids grow and that the typical form already noted becomes increasingly evident.*

Passing now to the unbroken portion of the chain, we find that the zooids increase in length very slowly at first and more rapidly later; also that though the curve is fairly smooth at first, it becomes quite irregular toward the end. Upon closer examination of fig. 2 and the graphs of other chains, we surmise that these irregularities are the forerunners of the groups making up the wheels; in other words that the periodicity shown so plainly in the wheel part of the chain extends back into the unbroken part. Were this found to be true, the fact could hardly be ignored in considering the problem of the break-up of the chain and the production of wheels.

In order to test the conjecture more critically we submitted the measurements to Mr. George F. McEwen, the mathematical expert of the Marine Biological Station of San Diego for examina-

tion. Out of this examination has come the graphs shown in figs. 4, 5, 6, 7, and 8.

A curve was computed to fit the graph (fig. 2), as nearly as possible. From the equation of this smooth curve we get a 'calculated value' for each zooid; that is the length of each zooid, if the series were as smooth as our calculated curve. We next subtract the observed length of each zooid from the calculated length, and get a series of values, some plus and some minus according as the irregular graph went below or above the smooth curve. When we plot these plus and minus values above and below a horizontal line we have the graph fig. 7. It shows that the values follow the curve fairly well at first and then vary more and more; in other words, that we have a periodic curve of increasing amplitude.<sup>1</sup>

<sup>1</sup> Mr. McEwen gives the following summary of the method used: The sizes for each of the points corresponding to the numbers 45, 50, etc., to 90 were taken as the ordinates of a curve whose abscissae were 1, 2, etc., to 10. It was assumed that the above curve corresponded to an equation of the form

$$y = a + bx_1 + cx_1^2$$

and the most probable values of the coefficients  $a$ ,  $b$ , and  $c$  were computed according to the method of least squares. By substituting  $(2x - 8)$  for  $x_1$  in the above equation, the equation

$$y = a + b(2x - 8) + c(2x - 8)^2$$

was obtained in which, if  $\frac{1}{10}$  of the number of the point is substituted, will equal the computed value of the corresponding size. (This equation was used to calculate the corresponding values of  $y$ , which were used in connection with the observed values for computing the algebraic sum of the residuals and the probable error, for the purpose of determining if the equation was a proper expression for measured values of  $y$ .)

It was assumed that this equation, determined from the 10 points was very nearly the same as if it had been computed from the 45 actual points, and therefore represented the relation between the number and the average size of all the points. This assumption was verified in one case by including all the points and comparing with the result when only 10 points were used.

The observed values of  $y$  were subtracted from the corresponding computed values and these differences were plotted as ordinates against the numbers as abscissae, thus giving a representation of the deviation of the observed values from those given by the equation. These deviations are due to errors in the measurements, and to the fact that the assumed equation was not a true expression for the relation. As the error in measurement was  $\approx 0.1$ , it is evident that the deviations are due mainly to the latter fact.

The periodic character of these curves shows that the true law is a periodic fluctuation of increasing amplitude about a mean value increasing in a regular manner with the number of the point.

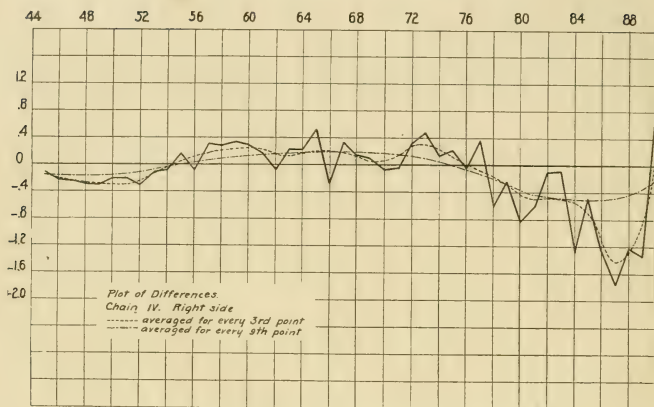


Fig. 4 Plot of differences for chain IV, right side.

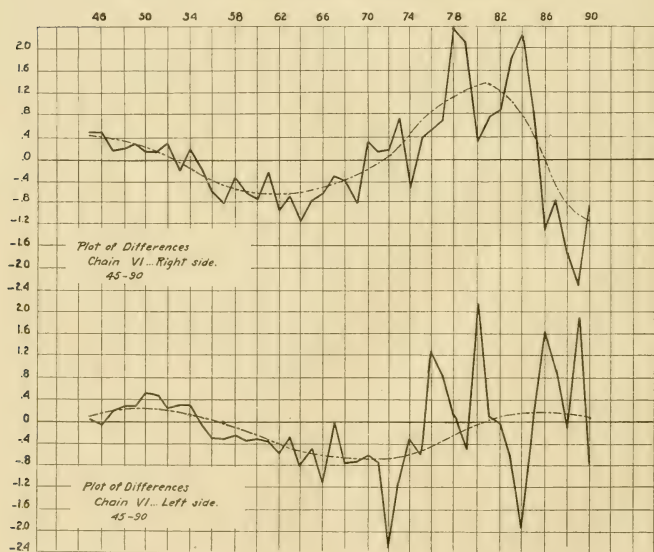


Fig. 5 Plot of differences for chain VI, right and left sides.

Chain IV, whose plot of differences is shown in fig. 4, is one of the smaller chains and in it one would expect to find the grouping less evident than in the larger chains. However it can be plainly seen even here. The right and left sides of Chain VI are shown in fig. 5. With Chains IV and VI, the differences were figured only for the zooids 45-90. In figs 6 and 7, the two sides of Chain VII

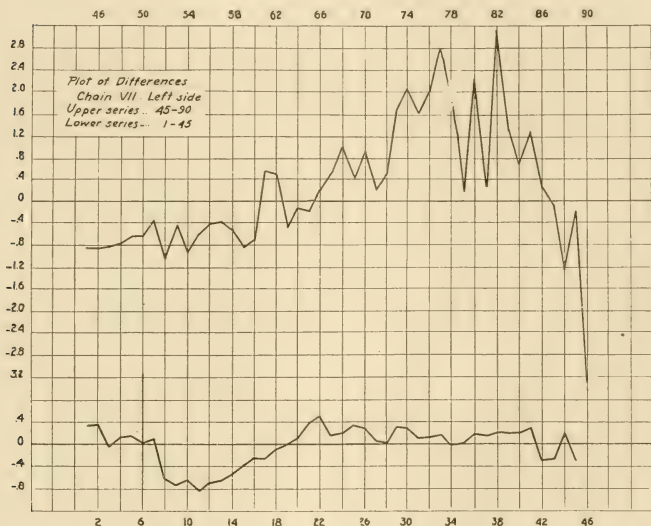


Fig. 6 Plot of differences for chain VII, left side

are given entire, the curves and the differences being figured separately for the two parts of the chain, since it can be fitted better when but half is considered at one time. The complete series being given, one can more readily see how the amplitude of the waves increases toward the end.

It will be remembered that in computing the differences, the observed values were subtracted from the calculated values. Hence upward curves in fig. 2 appear as downward curves in fig. 7. To make the comparison with the wheel graphs easier the signs

were reversed for fig. 8 so that values greater than the corresponding ones in the fitted curve lie above the  $x$  axis while smaller ones lie below. Vertical dotted lines have also been drawn to indicate a possible grouping of the zooids. Irregularities appear, it is true, but since irregularities often appear in the wheels also it is to be expected here. Moreover, with such small values the chances for error are so great that one would expect considerable variation.

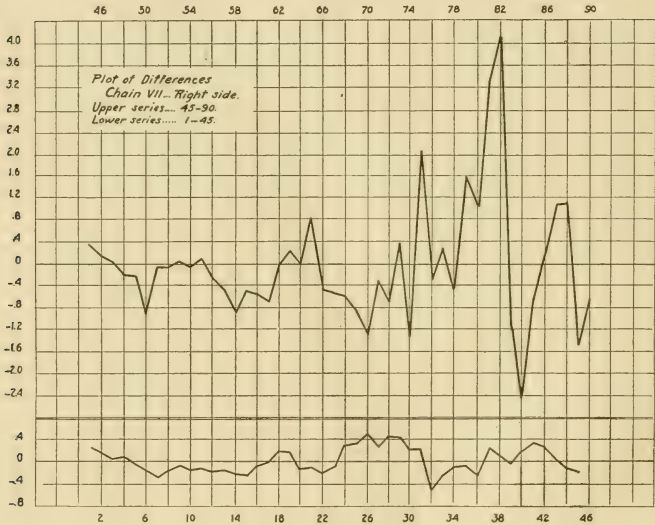


Fig. 7 Plot of differences for chain VII, right side

What we get then from these plots of differences is the *probable fact that the unbroken part of the chain really shows a periodicity or incipient grouping closely resembling that of the wheeled portion of the chain, the groups including four to eight zooids each, which is the number found in the completed wheels.*

The plots of differences brought to light an aspect of the matter which had not been anticipated, namely, the existence of *another wave with a longer period*, shown in all the curves. The plots in

figs. 4 and 5 have been smoothed by averaging for every ninth point in order to make this curve more plain. The curves are not just the same for the two sides of Chain VI, the difference probably being due to a difference in the way in which the computed curve fits in the two cases.

With what, if any, other biological phenomena in the species this newly discovered periodicity is connected we do not know.

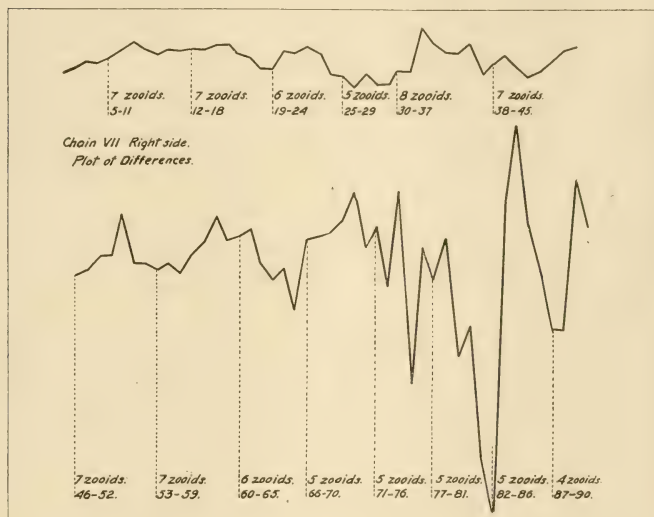


Fig. 8 Plot of differences for chain VII, right side  
Inverted and possible groups indicated

Of its existence however, there seems to be no doubt; and it is certainly interesting to recognize that we have here an instance, by no means uncommon in organic phenomena, of waves, so to speak, of one size riding upon those of another size. It is highly desirable to take these cases in hand with a view to finding their connection with other phenomena.

ATTEMPT TO CONNECT THE FORMATION OF WHEELS WITH MORPHOLOGICAL, PHYSIOLOGICAL, AND MECHANICAL PHENOMENA PRESENTED BY THE ANIMALS

1. *Segmentation of stolon and the deploying point*

To find other factors entering into the wheel production, a study of the structure of the chain was made. The portion of the chain in which the zooids are in single file is of the same general form as that of other species. The incipient zooids, marked off by the infolding ectoderm, have their aboral ends uppermost, and the dorsal side of each against the ventral side of its neighbor, the dorsal sides being towards the base of the stolon. The blood supply passes out through one-half of the large axial blood vessel and back through the other half. The segmentation of the stolon in some cases extends to the root of the stolon, in others not quite so far. A possible significance of this variation will be pointed out in another connection. Where segmentation extends to the root of the stolon, the more proximal segmentation lines are very irregular and this fact may be of considerable interest in a way we shall not stop to consider here. Judging by some chains, one would say that the segmentation begins at the sides of the stolon, but others lead us to suppose the beginning is above and below (along the genital rod and the neural tube) while in still others it seems to be equally advanced in all parts of the circumference. The latter condition is probably the usual one.

2. *Position and relation of the zooids in the chain from the deploying point to the twist*

a. *Shifting of the zooids.* What we call the deploying point, is the point, or region where the zooids, by moving alternately to the right and left shift from single to double file. While the zooid is moving out, it also moves upward and begins to turn, so that its dorsal side faces out instead of toward the base of the stolon. These changes take place gradually. The oral ends shove out and begin to turn and before the turn is complete, the aboral ends

shove out and turn. The sketch of the deploying point will make this clearer. Figs. 14, 15 and 16 are dorsal, lateral and ventral, views of the deploying point of one chain. These drawings were outlined with the aid of the camera and much care was taken to make them accurate.<sup>2</sup>

Calling the zooid whose oral end has just begun to shift, no. 1, the aboral ends of nos. 8 and 9 (numbering on one side only) are beginning to do the same. No. 25 (not shown in the figure) seems to have reached the final position with the rearrangement of the internal organs complete. We find now that the right sides of the right-hand zooids (considering those to be right-hand zooids that correspond to the right-hand side of the parent) and the left sides of the left-hand zooids are toward the base of the stolon. This statement applies to the chain before it emerges from the parent. The orientation of the older, extruded part of the chain is given later.

All of the observations on the early growth and differentiation of the chain agree with those made by Brooks for *C. pinnata* with the exception of the orientation. Brooks ('93 p. 79) says of the single file zooids:

At this stage each *Salpa* is bilaterally symmetrical, and its plane of symmetry is the same as that of the stolon, while its long axis is at right angles to that of the stolon, which becomes converted into a single row of *Salpae*, so placed that the dorsal surfaces of all of them are toward the base of the stolon, their ventral surfaces towards its tip, their right and left sides on its right and left respectively, their oral ends at its top or neural side, and their aboral ends at its bottom or genital side.

Again in his description of the double row he says:

The single row of *Salpae* becomes converted into a double row, which consists of a series of right-handed *Salpae* and a series of left-handed ones, placed with . . . the left sides of those on the right and the right sides of those on left towards the base of the stolon.

<sup>2</sup>The loop-like structures seen at the oral extremity of the zooids in figs. 14 and 15 might easily be mistaken for the intestine. They are not this structure, but indicate very nearly where the oral orifice will appear.

This, as will be seen by comparing it with our description, is the opposite of the condition found in *C. affinis*, since Brooks places the oral ends of the zooids uppermost while we find the aboral ends up. This mistake was probably due to lack of sufficient material for the study. He says (p. 87):

In all my preserved specimens the tip of the stolon had been so much flattened by contact with the side of the bottle, in transportation, that I have not been able to study in detail the way in which this wheel-like arrangement is acquired, and the subject should receive the attention of those who are able to study living specimens.

It is a point upon which one could easily go astray if hampered by a lack of material.

As the changes in internal organization seem to correspond with those of *C. pinnata*, and as Brooks' description is so clear and complete, we need not go into the subject, but refer to his account (Brooks, '93 pp. 80-106.)

When the zooid has moved into its secondary position it lies *upon* the stolon blood vessel rather than to the side or around it. With this change, two small vessels develop for each zooid, one leading to it from each half of the stolon blood vessel (fig. 24, *ibv.*). The blood flows along one-half of the main vessel (say the upper half) out through the upper small vessels to each zooid and returns by the way of the lower set of small vessels to the lower half of the main vessel where it joins the inflowing current. These currents are reversed with the reversal of the blood current in the parent. The zooids now increase in size very rapidly, lengthening out more above the upper level of the vessel than below it, so that at the twist the oral ends extend but a little way below the vessel, while the aboral ends extend far above it. As a result, the aboral ends of the zooids of opposite rows come in closer contact than do the oral ends. Since the zooids of the two rows are arranged alternately, each zooid will lie against two of the opposite row. As growth continues and the zooids, through their increased size, move outward as well as upward, they are forced farther apart, but the connection is retained through peduncles which now develop.

*b. The peduncles and foot-pieces.* These structures play so important a rôle in the production of the wheels that they must be described in some detail. Almost all the figures show the peduncle in one stage or another. Fig. 12, best gives its relation to the full grown zooid, showing that it is a thin flap or sheet extending out from the ventral median line. The diagram (fig. 17) shows that the peduncles of the series are parallel throughout the first part of the chain, and that each by means of its 'foot-piece' (*fp.*) is in contact with four others, its two neighbors in each row. These foot-pieces are also well shown in the right-hand portion of figs. 19 and 22. As the zooids grow and extend out farther from the blood vessel, the peduncles lengthen, and the foot-pieces grow longer as the zooids grow wider, at least until the region of the twist is reached.

Along with the great increase in the size of the zooids and the development of the peduncles comes a change in the circulatory system. The two individual blood vessels coalesce to form one vessel with two channels (fig. 24, *ibv.*). The blood current has the same course as before except that the incoming and outgoing currents of each zoïd pass through one vessel. Observation of the blood currents in the living animals made the task of working out the circulation much easier and more certain than it would have been if confined to preserved specimens. The cross section (fig. 25) shows well the relation of the zooids to the blood vessel, the foot-pieces joining the zooids above the vessel, (*fp.*) and the individual vessels leading from the zooids to the two parts of the large vessel (*ibv.*)

*c. The emergence of the chain to the outside world.* Through the first part of its course, the chain is enclosed within a definite tube in the test just below the endostyle, this tube ending at a point just posterior to the placental vessel and anterior to the first body muscle band. This first muscle bends posteriorly here so that its insertion is along the lower part of the tube opening. The placenta usually disappears before the chain reaches this point. There seems to be more or less of a cavity left in the test where the placenta was, and the chain, as it reaches this point, no longer being held in its horizontal position by the tube, following the

line of least resistance, turns down into the cavity, and by the rapid growth of the zooids, soon breaks through the thin wall to the outside, the tip bending downward.

d. *The twist in the chain.* The general character of this part of the chain may be seen from figs. 11 and 13, while the peduncles and blood vessels of the region are shown in the diagrams figs. 17 and 18. Before the twist, we have within the parent, a straight double row of zooids with oral ends down. After it, the chain is turned back under the parent, and the zooids are again found with oral ends down. Until the zooids break through the test to the outside the chain has not begun to twist, the zooids still lying symmetrically along both sides of the median line. In fig. 13 thirty-six zooids are outside and the twist is just complete. The presence of two rows of zooids in the chain makes the turn appear more complicated than it really is. The chain simply doubles back under and then turns over, this turn being almost invariably to the left. This leaves the zooids with aboral ends again uppermost, but the row that was before on the left is now on the right side of the parent.

e. *Reduction of foot-pieces, first break in the chain, and formation of the first wheel.* The first visible intimation of the break-up of the chain comes in the peduncles and foot-pieces. The foot-pieces (fig. 17) gradually grow longer toward the distal end of the chain, coming to their maximum length a little before the end of the unbroken part is reached. After the maximum they shrink (fig. 22). The decrease is much more rapid than the increase, there being only about sixteen to twenty-four zooids in the diminishing series. Fig. 19 shows that the first group consists of nine zooids whose peduncles have broken loose from the rest. The foot-pieces have shrunk still more and the distal ends of the peduncles have been drawn closer together. But while the foot-pieces, by which the zooids are held in the axial line of the stolon, become successively and rapidly smaller just before the beginning of the break in the chain, the zooids themselves are becoming constantly larger. A consequent crowding of the zooids results. This brings about a pushing of the bodies of the zooids forward in the chain beyond the foot-pieces. The strain to which

the series of adhering foot-pieces is thus subjected results inevitably in a pulling apart of the foot-pieces somewhere. As a matter of fact the break produces groups and not single pairs. These groups then promptly shape themselves into the wheels.

3. *Comparison of the rate of growth of the chain as a whole with the rate of growth of other animals.* As a matter somewhat to one side of the main problem, we have thought it worth while to compare the rate of growth of the chain as a whole with what is known of the growth of other organisms. This was done by the method employed by Minot ('91) in his study of the rate of growth of guinea pigs; namely, by finding the per cent of increase throughout the chain. The values of the corresponding zooids of Chains I, II, III, VI, and VII were averaged. (Chains IV and V being so much shorter were omitted.) The per cent of gain of the second over the first, third over the second, etc., was then computed and the values plotted. The result is a very ragged line showing a gradual increase through two-thirds of its length and a more sudden drop at the end. To get a graph whose course was more evident, the increment was computed again, this time taking the series in groups of five. The first value here is the per cent of increment of the second five over the first five, etc. (table 4, fig. 9). The gradual increase, maximum toward the end, and rapid decline is here plainly shown in spite of the limited data.

This result seems strikingly different from that for the guinea pig and other animals of higher order, where the per cent of increment is a diminishing one from birth on. The difference may, however, be more apparent than real since, to make the comparison more correct, it would seem that stages in the mammalian development preceding birth would have to be used.

The drop in rate of increase, when the wheel part of the chain is reached, may be significant for the comparison, but we do not consider our observations carried far enough into the life of the chain as a whole to warrant any speculation based upon them. A study of the growth of still younger and still older, larger zooids will have to be made to meet the requirements here.



Fig. 9 Percentage of increment throughout chain

## DISCUSSION OF THE OBSERVATIONS FROM THE CAUSAL STAND-POINT

### 1. Cause of the twist

Although salpae do not move through the water very rapidly, still there is enough motion to make the end of the chain double back under the parent, as soon as it projects into the water. The reason for its turning over is less evident. The zooids begin to pulsate some time before the twist is reached and, having been with aboral extremities uppermost in the original or normal position, we may suppose they tend to assume the same position again when the normal state of things is interfered with by the bending back of the chain. Observation of the living animals shows that the chains of wheels and the separate wheels (at least the smaller ones) usually move along with aboral extremities uppermost.

It is easier to say that zooids 'tend to assume the normal position' than to show the cause of this tendency. It may be that the specific gravity of the oral ends is greater, or that the pulsation may have something to do with it, or there may be some tropism

TABLE 4

*Per cent of increment throughout the chains*

SERIAL NOS. OF THE ZOOIDS	AVERAGE SIZE	PER CENT OF INCREMENT	PER CENT $\div$ 2
1- 5.....	6.5		
6-10.....	7.1	9.2	
11-15.....	7.8	9.9	
16-20.....	8.6	10.3	
21-25.....	9.4	9.3	
26-30.....	10.5	11.7	
31-35.....	11.9	13.3	
36-40.....	13.5	13.4	
41-45.....	15.2	12.6	
46-50.....	17.5	15.1	
51-55.....	20.1	14.9	
56-60.....	23.7	17.9	
61-65.....	27.6	16.7	
66-70.....	32.4	17.4	
71-75.....	37.0	14.2	
76-80.....	42.6	15.1	
81-85.....	49.7	16.7	
86-90.....	56.9	14.5	

*Wheel portion*

1-10.....	77.0	19.0	
11-20.....	91.6	19.0	9.5
21-30.....	101.5	10.8	5.4
31-40.....	117.5	15.8	7.9
41-50.....	134.1	14.1	7.1
51-60.....	149.0	11.1	5.6
61-70.....	165.0	10.7	5.4
71-80.....	183.7	11.3	5.7
81-90.....	200.6	9.2	4.6
91-100.....	221.6	10.5	5.2
101-111.....	236.9	6.9	3.5

involved; but we have no observations under this head. As the chain lies, with aboral ends of the zooids uppermost, the propulsion of the zooids drives them away from the ventral side of the parent, while if they were with oral ends up the pulsations would drive the oral ends up against the ventral side of the parent. In one specimen in which the chain had not yet emerged, the end of the chain was turning to get around the placental blood vessel. Had our observations been limited to this one instance, we might conclude that the twist is initiated in this way. But after looking over a large amount of material and finding that the placenta usually disappears before the chain reaches that point, it is evident that this in no way accounts for the twist.

2. *Unequal growth of zooids and foot-pieces as a factor in the breaking up of the chain*

We seem to have found a cause sufficient for the present research, for the break, *in some way*, of the chain of zooids. This is, as already pointed out, the unequal growth of the bodies and foot-pieces of the zooids. The first question that arises when we attempt to push the analysis farther is, why is the break into groups rather than into single pairs of zooids? Nothing in the differential growth recognized appears to bear upon this question. So far as that is concerned we should suppose the zooids would be picked off one by one, or at most in single pairs.

Just how constant these groups are, may be seen from the frequency polygon (fig. 10). We see that of ninety-two half wheels seventy-three contained six or seven zooids each, while only two contained eight, and five contained four zooids. This constancy is to be expected when we regard the breaking apart as a growth phenomenon depending upon constant causes rather than upon chance.

In some way the wheel phenomenon is clearly dependent to a large extent on the strength of the adherence among the foot-pieces, which are but parts of the central ends of the peduncles. As may be seen by fig. 18, the radial blood vessels, the other main connection of the zooids, break apart early in the life of the

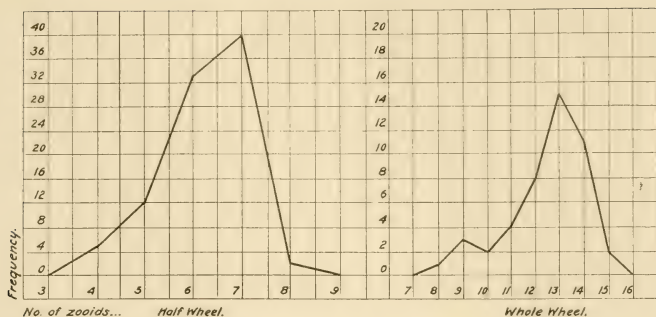


Fig. 10 Frequency polygon showing the number of zooids in the wheels

wheel, so that in the fully grown wheels the zooids are held together almost entirely by the peduncles. The observed facts certainly suggest group adherence among the foot-pieces themselves. Can direct evidence of any such thing be obtained?

Having proved the existence of a pronounced size grouping of the zooids in the wheels it naturally occurred to us that there may be something of the same sort in the peduncles and foot-pieces. We consequently made a considerable number of measurements on these structures. Some of the numbers are given in table 5, and in fig. 1; the graphs of peduncle lengths (dotted line) and foot-piece lengths (lower continuous line) are presented. It is doubtful if these show anything. We have not assumed that they do. The difficulties in the way of making the measurements are too great for the methods employed. It should, however, be borne distinctly in mind that these negative results prove no more than the insufficiency of the measurements. The fact that the foot-pieces do cling to one another in groups, and that the zooids to to which they belong are demonstrably different in size, appears to make it probable, *a priori*, that the adhesive power of the foot-pieces is of the gradational, or periodic sort, in spite of our failure to find it. The suggestion is that the graded size of the zooids is reflected in the adhesive power of the foot-pieces. Could this



63.....	18.0	17.0	20.0	18.5	18.0	18.3	18.0	21.8	19.5
62.....	18.5	18.0	19.0	20.5	19.0	17.0		21.5	21.5
61.....	17.5	16.5	19.0			17.0		19.8	18.5
60.....	16.5	16.5							
59.....	17.0	17.0							
58.....	13.5	15.5							
57.....	12.5	12.5							
56.....	12.0	15.0							
55.....	12.0	15.0							
54.....	11.5	15.0							
53.....	13.5	14.5							
52.....	14.0	15.0							
51.....	14.0	14.0							
50.....	13.0	14.0							
49.....	14.0	13.5							
48.....	13.0	12.5							
47.....	12.5	14.5							
46.....	13.5	12.5							
45.....	13.0	13.5							
44.....	12.5	12.5							
43.....	13.0	13.0							
42.....	13.0	13.0							
41.....	12.0	12.0							
40.....	13.0	13.0							
39.....	11.0	12.0							
38.....	12.0	11.0							
37.....	11.5	12.0							
36.....	11.0	12.0							
35.....	11.0	12.5							
34.....	12.0	11.0							
33.....	11.0	11.0							
32.....	10.0	10.0							
31.....	10.5	10.0							
30.....	10.0	10.0							
29.....	9.5	10.0							
28.....	10.0	9.5							

conjecture be proved true, an exceedingly important biological point would have been made.

And now as to the evidence that a periodicity corresponding to the future wheels does exist in the chain before its break-up. In discussing the results of our treatment of the data pertaining to the unbroken part of the chain, we said the curves, as shown in fig. 2, for example, 'probably' show a periodicity. We permitted ourselves to doubt to this extent, in the interest of conservatism. We wish now to sum up the evidence for periodicity. Its strength lies in the fact that it is cumulative rather than in the sufficiency of any one piece.

In the first place, does not the undoubted fact of periodicity in the wheels themselves, and the groups that immediately precede them, make the presence of periodicity in the rest of the unbroken part of the chain probable *a priori*? It would seem so. In the second place mathematical treatment of the quantitative data makes it almost certain that a periodicity corresponding to theory actually does exist. Third and finally the probable extension of the periods far back into the young part of the chain, leads us to suspect that this fact is connected with another observation of quite a different order, an observation, that is, which strongly indicates that the periodicity is really established at least as early as the segmentation of the stolon itself.

One of us has shown that in *Salpa fusiformis-runcinata* the very early segmented part of the stolon may be interrupted by an unsegmented part (Johnson, '10, p. 154 and fig. 8). While such interruptions have not been observed in *Cyclosalpa affinis* attention was called, when speaking of the first stages in the segmentation of the stolon, to the fact that in some cases the segmentation reaches to the very root of the stolon, while in others a stretch of unsegmented stolon exists. May not this difference indicate a periodicity in the segmentation corresponding to the periodicity in growth that we have found?

The reader may think that the grouping, as shown in the plots of differences, is too variable and indefinite to warrant the conclusions we have drawn. True, the groups here are not as regular as the wheel graphs shown at the end of the curve (fig. 2), but though

the small groups appear to be more irregular on account of their riding on the secondary waves, they are of the same sort. It must be remembered, too, that the values are very small and the chances of error are large. In fact, such a uniformity of result throughout all the graphs examined, in *spite of small values and difficulty of measurement*, is very convincing.

The transformation of the groups of zooids into wheels is easily understood: The moment the break occurs so that the pressure of the zooids upon one another in the group can exert its effect backward as well as forward, the hindmost pair swings in toward the axial line, each of the other pairs up to the transverse middle line of the group following in its proportional amount. Since by this time the foot-pieces have wholly or almost wholly disappeared and the central ends of the peduncles have become closely appressed, the swing of the zooids disposes the peduncles in the form of the spokes of a wheel, the hub being represented by a small elliptical space. The course of things here described is illustrated in fig. 17. That the pressure tending to force the mid-zooids of the groups outward is considerable is obvious from the zig-zag form into which the axial vessel is thrown, due to the pull on the radial vessels, as seen in the second group of fig. 18. The disappearance of the axial vessel in the older wheels may be supposed to be partly due to the same cause, although probably the vessel is actually in course of degeneration.

3. *Impossibility that the character of the blood supply to the zooids can be the cause of the size schemes within the wheels*

No study involving the growth of the zooids could be complete without attention having been given to so fundamental a matter as that of the blood supply. For example, the question naturally arises, does not the break-up of the chain into groups so affect the common blood vessel of the stolon that the zooids do not share alike in nutriment received, and is not this inequality responsible for the disparity in size among the zooids?

The changes in the circulatory system are best shown by the diagram fig. 18. At the end of the continuous part of the chain,

the individual blood vessels are arranged at regular intervals along the large vessel. The arrangement is the same for the first wheel, but with the second or third wheel the axial vessel begins to shrink. As the vessel remains in connection with the individual lateral vessels, while growing smaller, it comes to have a zig-zag course, due to the opposite but alternate pulls upon it by the growing zooids. The shrinkage of the vessels goes on so rapidly and to such an extent that in the next wheel the vascular connection between the central zooids is lost. The portion of the main vessel which joins two wheels together persists for some time. In fact this and the transparent cellulose envelope which forms around the wheels, filling in the spaces between the zooids, are all that hold the chains together and very long chains of wheels are sometimes found. The small remnants of the individual vessels gradually disappear. Though these vessels end blindly, the blood may still be seen in them for some time, flowing out one side and back the other. After the disappearance of the main vessel at the center of the wheels, short circuits are maintained between the zooids connected at any point. Thus in fig. 18, zooids 1, 2, 12, 6', and 7' have a circuit of their own. Thus it would seem that if any of the zooids of the wheels have an advantage over others the end ones would be favored as against the middle ones, but the middle ones are on the whole larger. Hence inequality in blood supply seems to be excluded from being a determining factor in the size relations observed.

If there be any communication between the zooids of the unbroken chain or of the wheels, other than by the circulatory system just described, it must be through the peduncles. The vessels in the peduncles are irregularly arranged but they are distinctly larger toward the edges and reach part way into some of the papillae. They are easily followed in the living specimens. To test the question of blood communication between zooids, injections were made. Methylene blue in sea water was used, which could plainly be seen in the transparent peduncles and in the bodies of the salpae. The first attempt was on two wheels whose stage of development was the same as the third and fourth in fig. 18. The needle was inserted in the stolonial vessel half way

between the two wheels. The color shot out through the small vessels to the peduncles of the zooids still remaining in contact. It went throughout the vessels of the peduncles of the zooids but stopped cleanly at the edge of the peduncle. No zooids whose connection with the main vessel had been lost showed any touch of the color. However, the wheel was again examined about fifteen minutes later. The stained zooids had died and dropped away from the wheel, the peduncle dropping away with the zooid. A slight stain was found around the papillae of the peduncles of one or two of the other zooids where they had come in contact with the stained ones. We conclude that there is no direct vascular connection here but that there is possibly some interchange by absorption through the thin ectoderm. Another injection was made in the peduncle of one of the zooids in a wheel. The color flowed throughout the peduncle and into the zooid but did not enter other zooids of the wheel. We therefore seem driven to conclude that the *blood supply is not a factor in the size differentiation of the zooids of a wheel.*

4. *Unlikelihood that the wheel arrangement of the zooids in Cyclosalpa has, as believed by Brooks, anything to do with the position of the four first blastozooids of Pyrosoma*

Brooks was firmly convinced that the radial, or wheelarrangement of the asexually produced zooids in Cyclosalpa is homologous with the radial disposition of the first four blastozooids of Pyrosoma. This he regarded as one of the strongest evidences of the close relationship between the two genera. Thus he says (Brooks '93, p. 133):

The opinion that Salpa and Pyrosoma are closely related does not however, rest upon superficial resemblances, but upon their fundamental identity of structure, although one of the details, the resemblance in their asexual multiplication, is so complete as to be almost enough in itself to establish their affinity.

The same view he expresses with only a little less assurance in several other connections. We had no thought, in entering upon

the present study, of considering this point, nor do we propose now to go into it extensively. However, our results on the growth and mechanical factors involved in producing the wheels of *Cyclosalpa* seem to have so much bearing on the question, that we can hardly pass it by without notice. The resemblance between such a figure of the *Cyclosalpa* wheel as, for example, that given by Brooks ('93, pl. 1, fig. 2), and reproduced by Delage and Hérouard (p. 203, fig. 151) and a figure of an early *Pyrosoma* colony like 15, (pl. 31), by Huxley ('59) is considerable and not unnaturally suggests true heredity kinship. The moment, however, one comes to look into the details of how each group comes about ontogenetically rather than phylogenetically, he finds them so different that his imagination is balked at an attempt to interpret them as both referable to a common hereditary operation. In the first place Brooks seems never to have observed the fact that the *Cyclosalpa* wheel is at the outset bilateral. None of his published figures give any intimation of this, nor does he refer to it in his text. For instance, the two figures, 8 and 9, pl. 2, of his latest publication (Brooks, '08) represent wheels of *C. floridana*, and *C. pinnata* as though they were perfect—as though the zooids were disposed in exactly the same way throughout the circuit. We would not, of course, assert that he did not draw just what he saw in these two instances, especially since we have had no chance to examine the wheels of *C. floridana*, and have seen but a single one of *C. pinnata*. In the one specimen of *C. pinnata* which we have, attentive study finds that two zooids on opposite sides of the circuit have slightly different positions from the others. These probably indicate where the axis of the chain lay; but the departure from perfect regularity is so slight and of such a character that it might be easily overlooked had one not discovered, by studying the formation of the wheels, what their real nature is. In *C. affinis* the bilaterality of the wheels is probably never wholly obliterated.

The first four ascidiozooids in *Pyrosoma*, on the contrary, stand in single file as do the *Salpa* zooids before the deploying point is reached and the radial order is taken on by the swinging around of the file so that number four comes to be adjacent to

number one. Further there is no opportunity in the Pyrosoma group for the differential mechanical action caused in Cyclosalpa by the growth and crowding of the zooids while the foot-pieces diminish in size. Neither is it possible seemingly, for the periodic phenomenon to play any such part in the arrangement of the Pyrosoma zooids as it appears to in Cyclosalpa.

#### THE LARGER SIGNIFICANCE OF SUCH STUDIES

##### *1. Supplementing biological with quantitative observations*

We venture to call attention to the way in which morphological and physiological observations and considerations join hands with quantitative observations in this research. Numerous structural details in the adult individuals of both sexual and asexual generations, in the chain of zooids as a whole, in the individual wheels and the individual zooids composing the wheels, and in the unbroken part of the chain both as a whole and as to its individual elements had to be attended to. On the functional side not only growth in several of its aspects, but the mode of swimming, certain facts pertaining to the circulation of the blood, and some points about nutrition have come in for consideration.

All this sort of thing is so familiar to modern biologists as to need no special mention. Not so with what we have done in a quantitative way. It seems to us that in this we have entered a region of research that biologists will be compelled to regard vastly more seriously in the future than they have in the past or do now. The case in hand furnishes a rather striking illustration of what the quantitative method can do. It can enable us to see facts we cannot see otherwise. It amounts to a great increase in the power of our eyes just as does the microscope. This statement is to be taken literally, not figuratively. One may easily imagine a magnifying instrument that would so enlarge the wheels as to make visible the size differences between the zooids. It would seem that this is what the application of mathematics in physical science very frequently does. We should never have suspected from ordinary examination size differences

of a systematic character among the zooids of the chains. It was only from certain biological considerations combined with aid from this instrument, that the existence of the system was made certain. And it should be specially noted how our results would have been affected by failure to recognize this fact. The breaking up of the chain *in some way*, and the production of wheels from the breaking, could have been inferred from the unequal growth of the bodies and foot-pieces of the zooids; but why the breaking should be *into groups* rather than *into single pairs* would have remained with no definite answer but for the discovery of the periodicity in growth in the unbroken as well as in the broken part of the chain.

## 2. *Natural periodicity in organisms and exacter methods of research*

But promptly comes the question from some of the foremost biologists, What of it? What particular good is there in knowing that growth is periodic so long as we have no explanation of why it is so? Our real interest, they say, is in the causes not the mere facts of organic phenomena. This objection displays, in our opinion, one of the most pervasive and fundamental weaknesses in the biological philosophy of the day. Looked at critically, it is found to mean that facts of nature, in order to be interesting and deemed really worth while, must be prejudged; that an explanation of them must be ready at hand before they are observed in order that they may be attractive. The issue must be looked squarely in the face. It is in fact the old, old issue between the inductive and the deductive methods of interpreting nature; between observation and reason going hand in hand, and the power of reason alone; between the *a posteriori* and *a priori* modes of reasoning. The objection carries the implication that great numbers of facts of nature can be explained without having been themselves examined; that the unobserved causes of many observable effects may be sufficiently inferred from observations on other effects than the particular ones under consideration. In a word the meaning is implied if not expressed, that some time nature may

be fully known without having been fully studied. This conception of nature and the knowledge of nature is always and everywhere the begetter of dogmatic assertion on the part of leaders, of subserviency to authority on the part of followers, and of idolatry to certain facts and neglect of others by everybody. This is not the place to go into the logic, or rather, the epistemology, of biology. The case under treatment does, however, justify us in a few observations and reflections on procedure in research.

Why is it that the biological sciences are designated as observational and descriptive, to distinguish them from the physical sciences which are called quantitative and exact? Surely no present-day student of nature would contend that living objects are qualitative alone and so must be dealt with in terms of quality, while non-living objects are quantitative and are to be dealt with in terms of quantity! There is surely no structural part or activity of any organism that does not exist in some quantity or other, and hence is not susceptible of being measured in some way. Contrarywise, there is surely no inorganic body or substance that has not qualities of some sort by which it is described and defined. Yet why is it that in spite of the brave effort made by a few distinguished men of science during the last half century to introduce conceptions of quantity and the methods of mathematics into biology, these efforts have met with only limited success at best, and are ignored in practice and frowned upon in theory by many of the foremost biologists? Only a few months ago a distinguished investigator declared in the presence of the senior author of this paper that the quantitative method in biology is dead, and this student suiting practice to theory, though working in fields where quantitative conceptions and exact determinations are particularly important, rarely attempts to measure in any rigorous way the biological phenomena with which he deals. Attention cannot be called too strongly to the extent to which much of what is esteemed the very highest type of recent biological work has laid stress on accurate quantitative determination of certain environmental factors of organisms, but has ignored almost wholly quantitative determinations of the vital phenomena themselves. There can be no question about the

importance of exactness in the determination of external factors. So far these methods are admirable; but, it appears to us, it must be recognized that when exactness has gone thus far it has gone at best not more than half the way. Nothing less than equal exactness all along the line will do to fulfil the highest demands of physical science.

Let one recall the degree of refinement with which physicists and chemists are measuring the phenomena with which they deal: the wave lengths and angles of refraction of light; the quantity of heat generated in chemical reactions; diffusion rates of gases and liquids; atomic weights and combining ratios, and innumerable other things. Then let him compare these with the ridiculously crude quantitative determinations made in nearly all departments of biology. A few aspects of physiology, as for instance, the temperature of the human body; and a number of phases of the psychology of higher animals—reaction times, for example—have been brought under mensurational treatment comparable with the standards of exactness long demanded in physics. But the vast fields of morphology, of general physiology, of individual and race growth and decline, of propagation, of variation, of automatic and responsive action, etc., have hardly been touched quantitatively as physics and chemistry would understand this term. As yet we in biology have hardly heard of anything corresponding to physical constants, units of measurement, coefficients of change, etc. Yet will any one, fully alive to the spirit of modern physical science, venture to maintain that inorganic phenomena are so utterly different from organic, that conceptions and practices so enormously fruitful in the one realm are wholly inapplicable in the other?

It is a significant fact that many biologists, the most ardent in defence of the so-called mechanistic or materialistic view of living things, are farthest away from, even most hostile to, the very methods for biology proper that have so largely made the physical sciences what they are. One looks in vain through numbers of technical writings by biologists of this school for anything like exact, comprehensive accounts, either qualitative or quantitative of organisms or parts of organisms, or even functions

of organisms, dealt with. Yet how these writings bristle with such expressions as 'differs considerably,' 'constant results,' 'as a rule,' 'very similar,' 'normal segmentation,' 'normal nuclear spindle,' 'normal blastulae,' 'normal animal,' 'practically identical,' 'essential features,' 'increases in exact proportion,' and so on!

Two rejoinders are frequently made to this demand for carrying more exact methods into biology. One is on the purely theoretical ground that it is not necessary; that 'mere quantity' is of no great moment in life phenomena; that slight differences are of the purely 'fluctuating' or individual sort, so have no large significance. To answer this objection in full would take us much farther into philosophical discussion than we can go here, but it may be the more warrantably passed by because the attitude of mind that makes it is seen to be obviously hostile to the whole trend and spirit of physical science. If the history of progress in science can be relied upon to furnish any clue as to how progress is to be continued in the future, the man of science, who holds a general view of nature that makes many facts insignificant and negligible, is bound to come to grief sooner or later.

The other objection is more practically justifiable. It is that the phenomena of living beings are so complex and subtle, and that animals, especially, are so sensitive to changes in external conditions as to make it impossible to apply to them in more than a very limited way, the exacter methods of the physical laboratory. Our answer to this is two-fold. In the first place, we are persuaded that exact methods could be applied far more widely than they are, and they undoubtedly would be, did our general conceptions call for such applications. The other answer is that if it be true, as it well may be, that many life processes are too subtle and involved to submit to measurement on an exact and large scale, then the only course open for the interpretation of such processes is to *introduce no considerations that involve the conception of accurately measured quantity*. The extent to which this principle, seemingly so obvious and unescapable, has been violated in much biological theory during the last quarter century or more, is seen to be remarkable once

one comes to think about the matter. For example, reflect on the extent to which theories of development and heredity have made use of the notion of equation and reduction nuclear divisions of the germ cells; yet who has determined in any rigid quantitative way the elements that enter into the hypothetical equalities and inequalities? How familiar is the textbook statement that the chromatin of the male fertilization nucleus is 'exactly equal' to that of the female nucleus with which it fuses! But on what sort of determinations does this assertion rest? On scarcely another thread of evidence than that they 'look equal!' And here we come upon the almost incredible naiveté with which biologists in most things eminently sound, have gone down before this fallacy! Only a short time ago while discussing this point with a number of biologists, one of them, a man of excellent standing and great carefulness in nearly all scientific matters, replied to my strictures, "if chromosomes look equal why are they not equal?" The words were hardly off the man's tongue when he saw what a remarkable statement he had made. The incident illustrates the straits to which one may blindly go in following a theory.

We conclude this topic with a quotation from John Tyndall. In his well-known address on the "Scientific Use of the Imagination," he says:

Let me say here that many of our physiological observers appear to form a very inadequate estimate of the distance which separates the microscopic from the molecular limit, and that, as a consequence, they sometimes employ a phraseology calculated to mislead. When, for example, the contents of a cell are described as perfectly homogeneous or as absolutely structureless, because the microscope fails to discover any structure; or when two structures are pronounced to be without difference, because the microscope can discover none, then, I think the microscope begins to play a mischievous part.

In view of the vast amount of evidence now before us from so many aspects of biology, that vital processes are periodic in their most fundamental manifestations, it appears unwarrantable to assume without proof that any whatever are not so. But see what periodicity means; it means that the phenomena are increas-

ing and decreasing; that they have phases; that the time element being considered, they change in value from moment to moment. How then can we treat any particular phase, or stage of such phenomena so as to meet the demands of rigorous science without considering each phase in relation to the other phases? So far as they are treated without such reference the procedure would seem to be of the nature of 'random observations'—of the 'grab-sample' kind—that always, whether in common life, business, or science finally proves to be inadequate if not disastrous. Astronomy, physics, chemistry, and in general geology, have passed quite out of this portion of their careers.

Taking it as established that biology is allied in essential nature with these older, less complex sciences, does it not seem inevitable that it too must move on and leave its cruder, haphazard methods behind? Does it not look as though this very fact of periodicity, this gradual come-and-go of things in the operations of organisms is to be one of the chief if not the chief way out? To press the inquiry a little closer, does it not look as though the wide prevalence of repetitive parts in reproduction and growth, which though like one another still differ from one another by some regular quantity, is to be one of the most important, though only one, of these exits?

It appears to us that cell division, for example, including the division of all cell parts subject to this process will have to be looked at sooner or later from this standpoint. Take the Foraminifera, for instance, unicellular organisms (according to the current interpretation) the bodies of great numbers of which become divided into many sections called nodes and chambers. In the great majority of species, as a glance at figures enables one to see, these divisions fall into quantitatively differentiated series. To make the point more cogent we introduce figures of two species *Reophax membranaceus* Brady (fig. 21) and *Peneroplis arietinus*, Batsch. sp. (fig. 20). Now let one compare these organisms with the salpa chain, the one, for example, represented in fig. 18, and catechise himself something like this: surely there is some resemblance between these objects. Both are composed of a considerable number of sections rather regular in form and much like one an-

other, though obviously differing from one another in size. Both objects are living, and both have come to be what we see them by a process of organic growth. Can we properly ignore these similarities in our efforts to interpret the organism, because on the whole the differences between them are more numerous and conspicuous than are the resemblances? Is it not at least possible that by turning to these few correspondences seriously they may serve as the starting point for the discovery of still others, and finally result in the detection of laws of organic growth and functioning that would greatly broaden our conceptions of, and hold upon, life phenomena?

One reason for selecting the Foraminifera as a group with which to make the comparison is the fact that the comparison of these organisms with higher ones in somewhat the same way has been made by several other zoologists. For instance, Schaudin ('95) speaks of the production and breaking off of parts in *Calcituba polymorpha* Roboz. as having "eine gewisse Ähnlichkeit mit der Strobilation."

But the most interesting comparison from our standpoint, of Foraminifera with other organisms was made by L. F. de Pourtales in 1850. At the meeting that year of the American Association for the Advancement of Science Professor L. Agassiz presented a short communication from this young zoologist in which Agassiz said:

Mr. Pourtales has, for the first time, pointed out a direct, well sustained analogy, which is to be found in the order of succession of the cells in foraminiferae of the genera *Textularia*, *Candima*, *Biloculina*, *Triloculina*, and *Quinqueloculina*. This succession agrees fully with the succession of leaves in plants—so fully that it can be expressed by the same fractions with which botanists are now in the habit of expressing phyllotaxis in the vegetable kingdom. This is, therefore, an important additional link in the investigation of the plan which regulates the normal position of parts in organized beings—a link which may lead to include into one universal formula the rhythmic movements which preside over the development of all finite beings. (Pourtales, '50, p. 89.)

This communication appealed strongly to at least one of those who heard it. At the next meeting of the association the presi-

dent, Professor A. D. Bache, said in what we should now call his presidential address:

The germ of two most important discoveries in natural history was contained in papers by two of our youngest members. [The first is omitted as not relevant.] The contents of the other were thus expressed: 'The order of succession of parts in foraminiferae is identical with the successive development of leaves in plants, and can be expressed by the same formulae.' Such discoveries, just warm from the study, it may be, as in these cases, forced to light by the occasion of our meetings, are among our greatest triumphs in the way of advancement. (Proc., vol. 4, p. 159.)

We find no evidence that these ideas of Pourtales have been carried farther either by him or by any one else, though our examination of the literature with reference to the point has been far from exhaustive. D'Orbigny, twenty-five years before, had done much work on the fundamental types of growth in the foraminiferae, though we find no reference to his having compared the arrangements here found, with phyllotaxy in plants.

Our object in calling attention to this matter is, in the first place, to show that we are not quite alone in thinking such comparisons are profitable; and in the second place, to call attention to the possibility that exact studies in the quantitative relationship existing among the members of a repetitive series as well as upon the ordinal arrangement of these members, may be profitable. But should it be found that such studies are significant when prosecuted on unicellular organisms in which the segmentation does not go to complete severance of the pieces, it would seem to follow that they should also be significant when made on species in which the severance is complete, and then to all cell division whatever.

This, of course, brings us immediately to the cyclical phenomena in the propagation of the Infusoria that has received so much attention in recent years, particularly at the hands of Maupas, Calkins, Jennings, Woodruff, and others. Concerning these researches we do no more now than remark that if the general conceptions on which we are going are sound, the phenomena of

protozoon division, and of all cell division will have to be examined much more systematically and vastly more exactly, quantitatively, than they yet have been.

3. *The inadequacy of treating periodicity generally, as an aspect of fluctuating variation*

Here seems to be the place to point out how much more objective, more workable, more important 'periodicity' is in our conception than it is as usually conceived by biologists.

We compare our ideas on the subject with those held by only one other investigator. Hugo de Vries has dealt with certain aspects of periodicity exhibited by plants, quite at length and in several of his works. He states the general facts with clearness. (De Vries, '05, p. 721):

This law of periodicity involves the general principle that every axis, as a rule, increases in strength when growing, but sooner or later reaches a maximum and may afterwards decrease. This periodic augmentation and declination is often boldly manifest, though in other cases it may be hidden by the effect of alternate influences. Pinnate leaves generally, have their lower blades smaller than their upper ones, the tallest being seen sometimes near the apex and sometimes at a distance from it.

There can be no doubt that the phenomena we are dealing with in *Salpa* and calling periodicity resemble closely those in plants thus described. The question, are they 'exactly the same' phenomena, we do not raise. Rather, we ask, in view of the closeness of resemblance ought they or ought they not to be looked at from much the same standpoint? The truth is de Vries has regarded the phenomena in plants very differently from what we have in *Salpa*, and his standpoint is surely inadequate for the facts we are dealing with. "This dependency on local nutrition," says de Vries, "leads to the general law of periodicity, which, broadly speaking, governs the occurrence of the fluctuating deviations of the organs" (p. 721). Again (de Vries, '01, vol. 1, p. 638) under the section, "Die Periodicität semilanter Eigenschaften," we read:

Ueber die grössere oder geringere Häufigkeit des Sichtbarwerdens semilanter Eigenschaften entscheidet nicht nur die augenblickliche Lebenslage, d.h. die äusseren Einflüsse während der empfindlichen Periode der Entwicklung. Fast ebenso gross ist die Bedeutung der individuellen Kraft des jungen Pflanzentheiles, diese aber ist das Ergebniss der Wirkung der äusseren Factoren in den vorhergehenden Zeitabschnitten, theils nach Wochen und Monaten, theils nach Jahren gerechnet. . . . Diese Erscheinung tritt am deutlichsten zu Tage in der Periodicität der Anomalien auf der Pflanze.

Again, pushing the matter a step farther, and in a somewhat different direction:

From a broad point of view, fluctuating variability falls under two heads. They obey quite the same laws and are therefore easily confused, but with respect to questions of heredity they should be carefully separated.<sup>3</sup> They are designated by the terms individual, and partial fluctuation. Individual variability indicates the differences between individuals, while partial variability is limited to the deviations shown by the parts of one organism from the average stature." ('05, p. 717). . . . The individual differences seem to be due, at least in a very great measure, to such apparent trifles. (As differences in soil, moisture, light, etc.). On the other hand partial differences are often manifestly due to similar causes. . . . The development of the leaves is dependent on their position, whether inserted on strong or weak branches, exposed to more or less light, or nourished by strong or weak roots (p. 721). Then follows the quotation already given, viz., "This dependency upon local nutrition, etc."

De Vries' standpoint seems clear: Periodicity in plants is a special form of the more general phenomenon of fluctuating variation which in turn is due to 'äusseren Factoren.' The quantitative differences that manifest themselves in the periods may be lumped together and treated according to the law of probability as first applied to organic beings by Quetelet. After illustrating the application of the method of statistics, the author says: "It should be repeated once more that the empirical result is

<sup>3</sup> It would be very interesting to have deVries follow up this point critically and impartially.

quite the same for individual, and for partial fluctuations" (p. 732). And: "In the present state of our knowledge the fluctuation-curves do not contribute in any large measure to an elucidation of the causes." (p. 734.)

And so we come to the real issue. Certainly, as de Vries says, the differences called partial *may be* treated en masse, so to speak. For example, we might pick to pieces ten wheels of the same dimensions of the *Cyclosalpa* chain, mix the zooids indiscriminately in a dish, then measure them and plot the results. The curve would be the same—the normal probability curve—but would give us no clue to the *way the zooids are disposed as to size in the individual wheels*. In that case the treatment would not, it is true, "contribute in any large measure to an elucidation of the causes." But in our case we have seen that no evidence can be found tending to show that the size scheme as it actually does occur in the wheels is dependent on external factors. All the evidence is to the effect that it is due to the growth process itself independently of any correspondingly differentiating external conditions. In other words, the periodicity in growth occurs under external conditions, that so far as the evidence goes, are not correspondingly periodic. Viewed in this light, can we still say the curves teach us "measureably little about the cause of the phenomena under consideration?" It seems to us not. Truly they do not furnish us 'a complete explanation' of the phenomena. They do, however, tell us, seemingly, this much: That the *cause is in the nature of the growth process itself*; that the growth goes that way.

If now it should turn out as suggested that not only the length of the zooids falls into a size scheme, but that many of the other morphological dimensions, and functional capacities fall into similar schemes, then the instructiveness of the curves would, for us at least, be very great touching the causes of the phenomena.

Whatever view may be held as to the relation of the periodicity in plants to that in the *Salpa* chain, it will we believe be allowed that the general question is one of many sides and great possible importance to biological theory. We have not pretended to do more than call attention to it here.

We conclude with an acknowledgment of our indebtedness to the work of several other biologists who have entered by one or another gate the course upon which we find ourselves. Of these perhaps the first to be mentioned is Julius Sachs whose idea of the grand period of growth in plants must, it seems to us, expand and play a much larger rôle in biological theory in the future than it has in the past. After Sachs, chronologically, the various investigations by C. S. Minot on the rate of growth in animals has largely influenced our observations and thinking. Another research, that by T. Tammes entitled "*Die Periodicität morphologischer Erscheinungen bei den Pflanzen*," has had considerable to do with shaping our ideas on the strictly biological side. But by far the most important as opening up the way to the quantitative work has been Raymond Pearl's "*Variation and Differentiation in Ceratophyllum*." Although Pearl's quantitative data in this research are entirely enumerative rather than mensural; and although his aims and results are in several rather important particulars different from ours, his fundamental problem really gave us our starting point.

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## PLATES

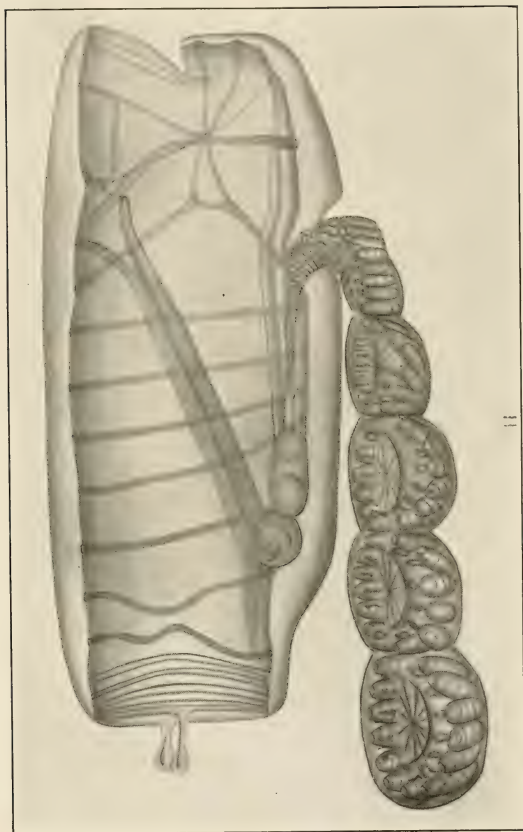
### ABBREVIATIONS

<i>atr.</i> , atrial orifice	<i>ht.</i> , heart
<i>emb.</i> , embryo	<i>i.b.v.</i> , individual blood vessel
<i>end.</i> , endostyle	<i>int.</i> , intestine
<i>f.p.</i> , foot-piece	<i>oes.</i> , oesophagus
<i>g.</i> , ganglion	<i>or.</i> , oral orifice
<i>gi.</i> , gill	<i>ped.</i> , peduncle
<i>gon.</i> , gonad	<i>ph.</i> , pharynx
<i>st.b.v.</i> , stolonie blood vessel	

PLATE I

EXPLANATION OF FIGURE

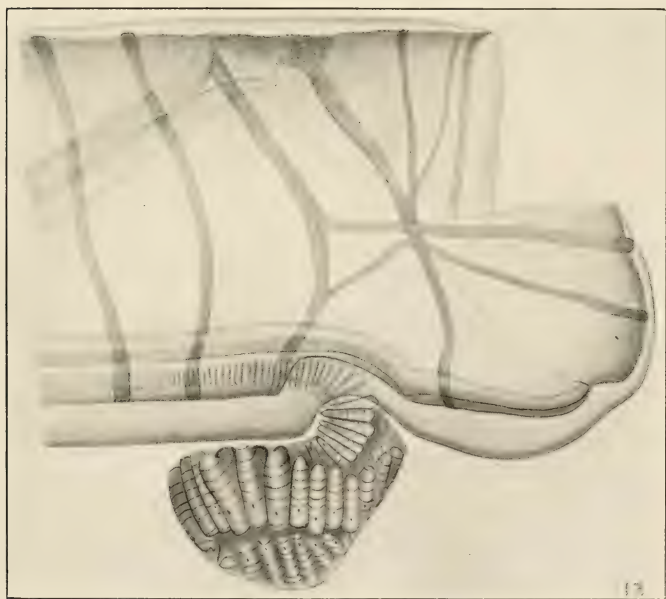
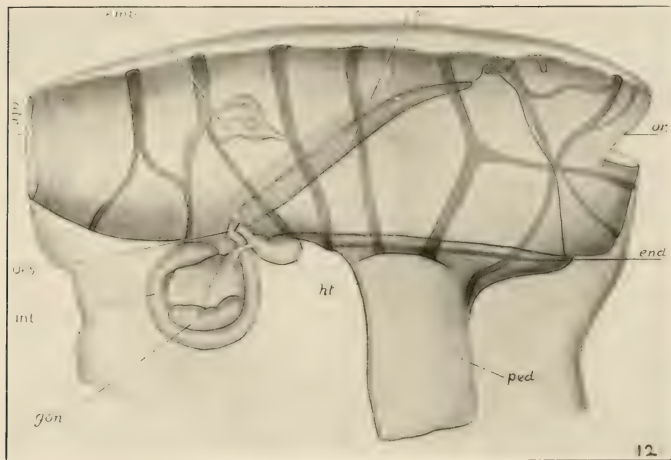
11 *Cyclosalpa affinis* Chamisso, solitary generation with chain of five wheels.  
Natural size.



## PLATE 2

### EXPLANATION OF FIGURES

- 12 *Cyclosalpa affinis*, aggregate generation.  $\times 1\frac{1}{2}$ .
- 13 *Cyclosalpa affinis*, solitary generation, with young chain of zooids just emerging.  $\times 2$ .



## PLATE 3

### EXPLANATION OF FIGURES

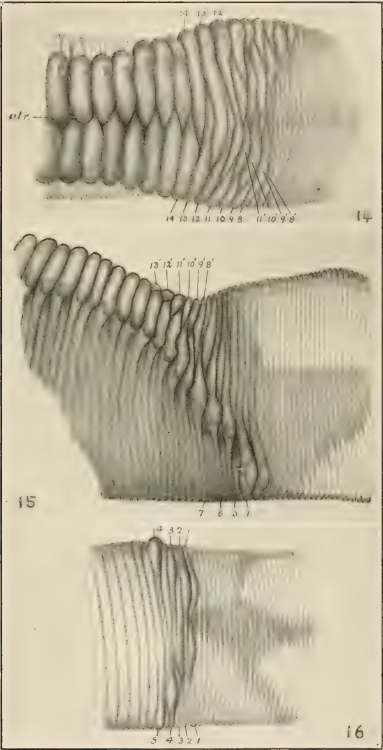
Deploying point of chain of *Cyclosalpa affinis*.

14 Dorsal view.

15 Side view, left side.

16 Ventral view.

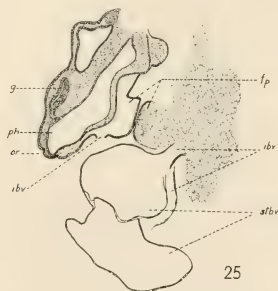
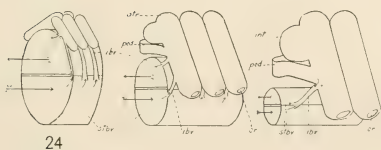
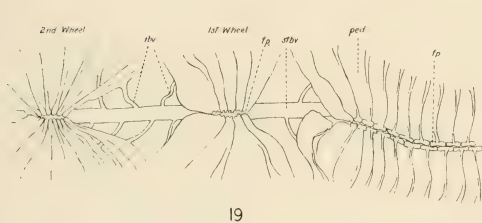
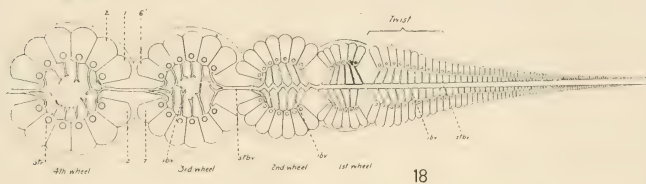
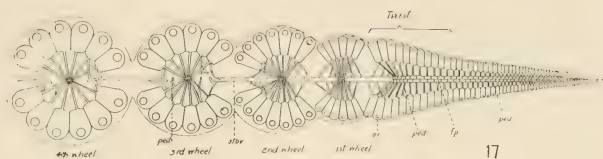
Zooids on the right side are numbered 1', 2', 3', etc.; those on the left, 1, 2, 3, etc. A given zooid has the same number in all three views.



## PLATE 4

### EXPLANATION OF FIGURES

- 17-19 Chain of *Cyclosalpa affinis*  
17 Ventral view of chain, showing unbroken part and four wheels. Somewhat diagrammatic but drawn to scale. Natural size.  
18 Dorsal view of same.  
19 Peduncles of distal part of unbroken chain and of first two wheels.  
20 *Peneroplis arietinus* Batsch, sp. Longitudinal section through the shell. Taken from Brady, Foraminifera, Challenger Expedition, vol. 9, plate 13, fig. 22.  
21 *Reophax membranaceus* H. B. Brady. Taken from monograph of the Foraminifera of the North Pacific Ocean, Cushman, 1910, U. S. Nat. Museum Bulletin 71, p. 90, fig. 126.  
22-25 Chain of *Cyclosalpa affinis*.  
22 Enlarged view of the distal foot-pieces of the unbroken part of the chain.  
23 Enlarged view of the foot-pieces of the first wheel.  
24 Diagrammatic representation of three stages in the development of the circulatory system of the chain.  
25 Cross section through chain.





# ON THE FORMATION, SIGNIFICANCE AND CHEMISTRY OF THE WHITE AND YELLOW YOLK OF OVA

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University of Chicago*

THREE PLATES AND ONE TEXT FIGURE

Introduction .....	455
A method of measuring the rate of growth of rapidly growing ova.....	457
The rate of growth of the ovum of the common fowl.....	458
1. Large ova, more than 6.0 mm. in diameter.....	458
2. Small ova, less than 6.0 mm. in diameter.....	459
The thickness of the strata of white and yellow yolk in the egg of the common fowl .....	461
The coincidence of the amount of yolk deposited in a day, with the amount of yolk contained in a layer of white and yellow yolk.....	462
Yolk stratification in eggs of other animals as seen in the light of its causation in birds.....	462
On the chemistry of white and yellow yolk.....	467
On the mechanism of yolk formation and de-formation.....	470
1. The part played by the reversible action of enzymes.....	471
2. The rôle of the partition coefficient of the elements of yolk .....	475
3. These two factors and the histological data .....	477
Summary .....	482
Literature cited.....	485

## INTRODUCTION

Very many thousands of pages have been written concerning yolk—its presence, formation, varieties and distribution in eggs. Indeed, the task of recording such a series of facts has been repeated on nearly every egg that has come under the closer observation of the biologist; while some eggs, notably those of the frog and the fowl, have submitted their yolks to the observation and description of dozens of different investigators. Not-

withstanding this great amount of study and description, the literature fails to give satisfactory answer to any of the following questions: (1) Precisely how and where does yolk originate? (2) Why, or how is it that there are two kinds of yolk, (a) smaller spherules (often with enclosures) of white yolk, and (b) larger spherules of finely granular (often pigmented) yellow yolk? and, what is the relation between these? (3) What is the meaning of the stratified condition of the yolk of some eggs, eggs in which layers of white yolk alternate with layers of yellow yolk? (4) What are the chief chemical differences between these two kinds of yolk?

Thinking that we are now able positively to answer questions 3 and 4, and that these solutions bring some light upon the first and second questions, we submit the following data and considerations. These are presented with a minimum of reference to the enormous literature; otherwise this communication must have been increased to several times its actual size.

In carrying out this work, and now in the presentation of it, the author would say that he has not forgotten that 'yolk' is 'non-living substance' and therefore from a certain standpoint has but a minor interest to biologists. But, standpoints change. Until Johannes Müller declared, and Van Beneden clinched the point, that the yolk of eggs is not living matter, and that it contrasts absolutely with the other part of the egg—the protoplasm—yolk had an all-absorbing interest to naturalists as a substance *per se*. In the years that followed, yolk has been studied largely with a view to cataloguing its diverse occurrences, forms, origins, distribution, tingibility, etc.; its interest to most students has flagged; though its often overweening bulk in the most studied of all cells has frequently won for it unwilling and tedious description. Perhaps one day we shall have a new standpoint. At any rate, we are only now beginning to realize that, though yolk is non-living substance, it is nevertheless *organized* substance and a very refined product of the vital laboratory; that it is a product laid down in the meshes of protoplasmic elements; and that the very act of its laying down is a signal of important metabolic states and capabilities of these living elements. More of the im-

port of the relation between this 'organized' and this 'living' material we shall know later; in the meantime, each bit of information is doubly welcome because it concerns the most interesting form of protoplasm—the egg—at what is probably its most interesting period. Perhaps, then, the substance that has seemed to have but blundered in where it could blind us most, may itself prove to be a mirror for many a secret that we have elsewhere sought in vain.

#### A METHOD OF MEASURING THE RATE OF GROWTH OF RAPIDLY GROWING OVA

The present studies began with an attempt to learn the *cause* of the stratified condition of the yolk of the hen's egg. It was suggested to me by results of an earlier study ('08) that the alternate layers of white and yellow yolk in the egg may be the result of the daily rhythm of nutrition—connected with high and low blood-pressure—which I had discovered in birds, and which I had shown to be the cause of the alternate fault-bars and fundamental bars of birds' feathers; it being there found that the daily variation in nutritive conditions in birds is sufficient to produce structurally perfect, and structurally imperfect parts in their rapidly growing feather germs. To test the suggestion, then, one might need only to learn the rate of growth of a bird's egg. What is the rate of growth in the eggs of the common fowl? This had not been determined, and no way of determining it was known.

It occurred to me that Sudan III might be used for this purpose. Knowing that Sudan was not destroyed in passing through the intestinal wall, (Daddi) that it circulated tied to the fatty acids of the food, and that the fatty acids of the food were laid down unchanged in the egg (Henriques and Hansen, '03), I inferred that Sudan given with fatty food would be laid down in the egg.

Moreover, it seemed possible by regulating the dosage and using proper intervals between feedings, to get laying hens to put this bright pigment down as definite bands in their growing ova, and thus enable one to determine the rate of growth.

The first experiment was as successful as the last. When such Sudan-containing eggs were hard-boiled and sectioned under

water it was easy to measure the distance between the innermost borders of two such rings of Sudan, and thus to identify this amount of growth with the time which was known to have intervened between the two feedings.

Having thus discovered a method<sup>1</sup> (described in detail by me elsewhere, '10) of measuring the rate and time of growth of ova, many data were collected on this point; the distance between the normal strata (layers of white and yellow yolk) of the egg was carefully measured; later the problems and considerations growing out of the results were further followed up. We give here the following short statement of the observations and conclusions:

The radius of the hen's egg increases during the last few days of its growth by about 2.0 mm. per twenty-four hours. The thickness of a layer of white yolk and a layer of yellow yolk taken together is usually about 2.0 mm. Our conclusion is that in the fowl a layer of white and another of yellow yolk are laid down each twenty-four hours. Other facts at hand indicate that the yellow yolk is laid down under the best nutritive conditions, while the white yolk is a sort of growth-mark left by poorer nutritive conditions.

#### THE RATE OF GROWTH OF THE OVUM OF THE COMMON FOWL

##### 1. *Ova of more than 6.0 mm. in diameter*

Table 1, section A, and plates 1 and 2 have been prepared to show the rate of growth of the larger ova as this is indicated by the Sudan method. The reader is referred to the table and plates in order to learn the kind of evidence on which the first conclusion is based. The amount of this evidence could be increased several times. It will be seen that the radius of the larger ova contained in the ovary of a fowl may increase by rather more than 2. mm. during twenty-four hours; also that this rate of growth is quite variable and may often fall to one-half the above amount.

Other data in our possession show that this rate not only varies for the eggs of different ovaries, but for different eggs of the same

<sup>1</sup> First announcement of the method, and of some of the present results, Riddle ('07).

ovary which were grown at different periods. It has been shown moreover by the Sudan-method that the rate of growth may be reduced not only to one-half that given above, but to absolute zero; this, however, is only a confirmation of what inference has long declared must be so, since ova may even decrease in size while in the ovary, *i. e.*, they may be resorbed.

TABLE 1

Showing under section A the rate of growth of hens' eggs as this was measured in central and peripheral parts of the yolk by means of Sudan. The numbers in the first column refer to the number which this egg bears in the plate. In section B are recorded measurements of the thickness of pairs of white and yellow yolk strata in central and peripheral regions of the ovum as this could be seen with unaided eye or with addition of iodine solution. The seven measurements here chosen arbitrarily from nearly forty in the records, are consecutive measurements of eggs from different hens.

A				B	
NO. OF EGG	24 HOURS RADIAL GROWTH IN MM.		NO. OF OVARY	THICKNESS OF A PAIR OF YOLK STRATA	
	Central	Peripheral		Central	Peripheral
5 Pl. 1		2.2	1 <sup>a</sup> Pl. 1		1.67
3 <sup>a</sup> 1		1.41	1	2.16	2.16
4 <sup>a</sup> 1	1.3	1.7	2	1.54	1.54
5 <sup>a</sup> 1		1.7	3		1.75
6 <sup>a</sup> 1		1.64	4	2.5	2.5
1 2	1.8	1.8	5	1.4	1.4
2 2	1.5	1.5	6	2.0	2.0
5 2		1.3	7	1.47	1.47
Average. ....	1.53	1.67	Average. ....	1.85	1.81

## 2. Ova of less than 6.0 mm. in diameter

It has not been possible to obtain a deposit of Sudan in eggs smaller than 6.0 mm. in diameter. This failure is explained by the fact that these ova are growing very slowly, as compared with the more advanced ova, and the intake of the stained food is here not rapid enough to give a perceptible effect. We shall see, moreover, that this white yolk—for ova of this size are com-

posed entirely of white yolk—is much poorer in fat than is yellow yolk. Since fat is the only food that can carry Sudan this is another reason for the failure of Sudan to appear in them. The Sudan method is therefore not available for the determination of the rate of growth in these eggs.

One bit of evidence of another sort concerning this rate of growth was obtained and may be recorded. In fig. 3, pl. 2, is shown the striated appearance which the peripheral white yolk of one of the small eggs showed after having lain in a quantity of Mann's formalin-alcohol mixture for a few weeks. Here the noteworthy facts are, that a striation exists, and that the lamellae are not thicker than 0.25 mm. Whether these lamellae are made up of still smaller strata which really represent days of growth I am quite unable to say. I doubt somewhat that the radius of these small eggs is increased by as much as 0.25 mm. in twenty-four hours; anyway these strata offer some evidence—in the light of what we know of succeeding yolk strata—that these small eggs do not grow *faster* than 0.25 mm. per day.

One must ask what is the meaning of the extraordinary difference in growth-rate of eggs under, and over, 6.0 mm. in diameter? What old mechanism is inhibited or what new one brought into action, that accounts for this procession of cells—each with *months* of slow and constant growth behind it—coming to a point from which each jumps in a *day* from its accustomed rate of increase, to a rate that is probably from eight to twenty times higher? Do the follicular cells now become more permeable than formerly to the ingredients of yolk? Is the increased vascularity of the follicular envelopes, that certainly occurs at this time, a cause or a result of the new activity? To these questions there comes no answer. But to us there are few events in the history of the primary oöcyte of the fowl more interesting than this one. All the more interesting it is, too, because of its glaring apparent teleology. Here is an ovum within five to eight days of extrusion<sup>2</sup> and containing less than the hundredth part

<sup>2</sup> It is true, however, that if the yolk grow less rapidly than normally the egg remains longer in the follicle; showing that the time of ovulation is not controlled by heredity but is governed quite completely by conditions.

of the yolk necessary to make it capable of producing an animal. Nevertheless five to eight days suffice to supply the missing ninety-nine parts.

THE THICKNESS OF THE STRATA OF WHITE AND YELLOW YOLK  
OF THE COMMON FOWL

The measurement of the thickness of a layer of yolk offers some difficulties and can rarely be done directly on a single layer; the reasons being that one stratum merges very gradually into another and that the strata are often very indistinct. More frequently, though by no means in every egg, a series of well-marked layers can be found and a measurement made over all; the number of strata—or rather of pairs of strata—may be easily counted. When the total measurement is divided by this number one obtains the thickness of a combined layer of white and yellow yolk.

The result of eight such measurements is recorded in section B of table 1. These are typical of nearly forty reliable measurements, and indicate a thickness of about 2.0 mm. (1.4 – 2.5) for a layer of white and yellow yolk combined.

The layers of yolk can sometimes be seen in the fresh eggs, proving that they are not artifacts; but for the purpose of measurement it is usually best to hard-boil them, and section (under water) from one side until the exact center of the egg is reached. Sometimes it will be found advantageous to put the egg thus prepared in weak iodine solution for a time. This treatment seems occasionally, though not always, to strengthen the contrast between the layers of white yolk and those of the yellow variety.

For reasons stated above it is impossible satisfactorily to measure the thickness of a layer of white yolk. It can be said with confidence, however, that this so-called layer has but a fraction of the thickness of the adjoined yellow layer. Perhaps one errs but little in saying that the former usually has from one-fourth to one-eighth the thickness of the latter.

THE COINCIDENCE OF THE AMOUNT OF YOLK DEPOSITED IN  
A DAY, WITH THE AMOUNT OF YOLK CONTAINED IN A  
STRATUM OF WHITE AND YELLOW YOLK

A comparison of the two sections of table 1 shows quite convincingly, I think, that the figures, which in the one column indicate the amount of a day's growth, are of the same order of magnitude as those which in the other column indicate the thickness of a stratum of yolk. *This fact, and another one, namely, that we know that there exists in birds a daily nutrition rhythm capable of producing daily growth-marks in their rapidly growing feathers, convince us that a layer of white yolk and another of yellow yolk is laid down during each twenty-four hours.*

The well-developed appearance of the yellow yolk, its large yolk-spherules and its much greater thickness than that of the white layer, all indicate, moreover, that this layer, like the broad fundamental bar of the feather, is grown under the best nutritive conditions; while the narrow layer of white yolk with its small spherules gives indication that it, like the fault-bar of the feather, is grown under poor nutritive conditions.

*Since I have shown that the poor nutritive conditions which produce the fault-bar occur in the later hours of the night—1:00–5:00 A.M.—I consider it as practically certain that the white yolk of the ovum is produced at the same time, and that the yellow yolk is produced during all other hours of the day.*

The layer of white yolk of the hen's egg is then a growth-mark left at the ever-changing boundary of the ovum; it represents the results of yolk formation under sub-optimal conditions. It is indeed incomplete, unfinished yolk, as is apparently indicated by the histological data already known, and by the chemical evidence which I shall present in another section.

YOLK STRATIFICATION IN OTHER ANIMALS AS SEEN IN THE  
LIGHT OF ITS CAUSATION IN BIRDS

With the story of the white and yellow yolk of the bird in mind it becomes most instructive to reëxamine many of the peculiar types of yolk distribution which from time to time have been re-

ported and figured by embryologists and cytologists; for now we can feel fairly sure that wherever we meet alternate layers of white and yellow yolk, such layers indicate just so many alternations of better and poorer nutritive conditions during the time these layers were being formed. The better and poorer nutritive conditions doubtless applying to the organism as a whole.<sup>3</sup>

A zonal arrangement of yolk similar to that of the bird has been reported in at least four other groups, viz., turtles, lizards, skates, and myxinoids. Some yolk patterns are known which are not distinctly zonal but intermediate to it and the type of yolk arrangement which is usual in small eggs; these help to bring all yolk distribution under a single principle or set of principles.

In order to avoid much tedious description in the text, and also to present more clearly and accurately this part of the subject, I have prepared plate 3, which is to a large extent a reproduction of figures which are not new. To what is shown in the plate, and in the explanation which accompanies it, I here add the following:

In all ripe ova, as in all the growth stages during which yolk is being deposited in the ovum, a layer of yolk composed of very small spherules (white yolk) is to be found at the extreme periphery of the egg. If larger yolk spherules (yellow yolk) also occur, they occupy more central portions of the egg. There is, moreover, scarcely an exception to the rule that the germinal vesicle or egg-pronucleus is immediately surrounded by similar small spherules and not by large ones.

It seems also to be very generally true that in those ova in which considerable yolk is developed, and in which the germinal vesicle makes its way from the center to the periphery of the egg (or remains near one side of the cell) it leaves in its wake a cylinder of white yolk to which in some cases has been given the name of Pander's nucleus.

All of these features are shown in eggs of such widely separated forms as the skate (fig. 6) the amphibian (fig. 5), the lizard (fig.

<sup>3</sup> On the other hand, some eggs, *e. g.*, those of the salmon, may undergo their chief growth at the expense of the somatic tissues and while no food whatever is being ingested. The conditions here, however, are essentially *constant* and therefore produce no stratification of the yolk.

8), the birds and at least in some mammals (fig. 3). These are the forms, too, which—with the exception of the mammal—in addition show a stratification of the main body of the yolk. Two other forms are known, the turtle and the cyclostome (*Bdellostoma*) in which the stratification and other features occur, as in the above-mentioned eggs, except that no Pander's nucleus has been found.

How may we explain at one and the same time the essential similarity of the yolk distribution in eggs of widely separated forms, and the often essential dissimilarity of its distribution in the eggs of closely related species? There seems now no doubt that all can be accounted for when one knows two things: first, the length of the growth period; and, second the chief fluctuations in the nutrition of the animal during the growth period of the eggs.

Most ova have no stratification, then, because the yolk is grown in a short season—the animal not being subjected to such severe alternations as winter and summer, while the process is going on; or, because the eggs remain very small and develop little yolk; or, again, because some ova have the extraordinary capacity of growing at the expense of somatic tissues. In such cases fluctuations in the nutrition of the *animal* are of little moment to the egg; the latter being able to feed well at the expense of the organism as long as it continues to live.

When stratification is present, however, I believe this to be a positive declaration that nutritive fluctuations did occur in the organism, and the number of the strata to be a reliable index to the number of such fluctuations. The presence of yolk stratification in the eggs of an animal then is an invitation to the naturalist and physiologist to look for important nutritional variations in that animal.

Thus far definite causal and time relations between such stratification and nutritional fluctuation has been determined only for the bird. What this time period is in *Bdellostoma* we can now only conjecture; but the fact that in a mature specimen eggs of a wide range of size exist possibly argues that these eggs are several years in forming. The further fact, that the animals lose much blood and become much weakened at each yearly spawning

period, is significant in that here may be found the means of a nutritional depression which produces a layer of white yolk in all of the remaining eggs of the ovary. If this be the true explanation one can readily understand the lack of stratification in the eggs of the related *Petromyzon* (fig. 2) since this form spawns but *once* in a lifetime.

In the skate the main growth period of the oöcyte is probably completed in less than a year. The nine or ten pairs of strata figured by Rückert (fig. 6) are probably produced at the rate of about one per month. Whether this refers merely to the number of times the animal has fed during this time, or otherwise, nothing seems to be known.

The amphibian egg has a short growth period, and derives its growth material too from substances stored in the body, and is thus independent of external food supply. Doubtless these facts—together with its usually moderate size—will account for the actual configuration of its yolk.

The eggs of two reptiles—turtle and lizard—show very evident, but dissimilar, yolk strata. What the time, or the nature of the nutritive fluctuations are, that may produce these strata in *Lacerta*, I can make no suggestion.

In the egg of the tortoise Munson ('04) seems not to have identified (fig. 1) the so-called inner and outer cytoceol as layers of white yolk. A study of my own preparations, however, convinces me that such is their nature and the term *cytoceol* therefore is unnecessary. The turtle's egg has then alternate layers of white and yellow yolk somewhat comparable to those of the bird. I have found indications of four pairs of such zones in some eggs; or rather, by comparing the strata of different eggs from the same animal I have found such indications. But I am not now sure that four such pairs exist, nor that only four exist. Certainly *several* very thin strata can sometimes be found within 2 mm. of the periphery of some ova.

One wonders much whether the well-marked innermost layers of the turtle's egg can be the indications of *years* of growth. Agassiz ('57) showed that these ova undergo their greatest growth in four interrupted stages extending over four years. Our predic-

tion is that further examination of these yolks, by proper methods for differentiating the strata, will show four pairs of white and yellow zones, to correspond to four yearly periods; each year supplying a period of growth and of rest, or at least of more rapid, and of less rapid growth.

Of the mammal's egg shown in fig. 3 it can be said that the several conditions of its growth seem to be closely similar to those of the amphibian egg which it so much resembles. To be sure, this egg may not, like the amphibian, develop at the expense of substances stored in the body; but, so few eggs are here developing at one time that an adequate food supply is always assured.

We believe then that these data practically give answer to the very important question which has been so well put by Rückert ('99, p. 585):

Diese Uebereinstimmung des Selachier—speciell des Torpedo-Eies, mit dem Vogelei ist, wenn der Vergleich sich zunächst auch nur für die gröbere Structur durchführen lässt, immerhin eine auffallende Thatsache.

Es würde die Mühe wert sein, bei einer erneuten Untersuchung der ohnedies seit vielen Jahren vernachlässigten Dotterentwicklung nach Anhaltspunkten zu suchen, ob die Aehnlichkeit nur dadurch hervorgerufen wird, dass die beiderlei Eier unter gleichen Bedingungen sich entwickeln, oder ob es sich um einen durch Vererbung auf das Vogelei übertragenen Vorgang handelt; mit einem Wort, ob eine Analogie oder Homologie vorliegt. Im letzteren Falle würde sich der Schluss ziehen lassen, dass das meroblastische Ei des Vogels resp. der Sauropsiden ein primär meroblastisches ist wie das Selachierei und das Säugetierei kein tertiär sondern ein sekundär holoblastisches wie das Amphibienei.

The similarity noted above of the amphibian and marsupial eggs is another case in point. My results indicate that the likeness of yolk distribution in these two eggs, and in those of selachian and bird cited by Rückert, *does not rest on heredity in any narrow sense of the word, but on the fact that they develop under like conditions.*

#### ON THE CHEMISTRY OF WHITE AND YELLOW YOLK

The conception of white yolk which arose from the preceding work was that such yolk is a halted, or intermediate stage, in the

development of yellow yolk. This same conception had been urged on histological grounds by several workers, though opposed by others. The chemistry of the two substances was then appealed to for further evidence of a sort which it alone could give.

An examination of the rather abundant literature on the chemistry of yolk showed that it contained none of the data which our problem required. Analyses of yellow yolk have indeed been made by Prout, by Gobley and by Parke; but it was believed that the extraction methods of their time did not effect a complete separation of the fat from the other constituents of the yolk. These determinations have therefore been made anew. That such was really necessary may be indicated by the fact that Parke ('67) extracted only 66.7 per cent of fat and phosphatids, whereas my analyses always yielded more than 70 per cent of these constituents. It was also imperative of course that results of analyses which were to be compared should be obtained by identical methods. Apparently no analysis of white yolk had been made, so that this had to be done.

Since, moreover, the metabolism of yellow yolk includes not only its formation but also its de-formation into absorbable constituents, it was considered necessary to take account of yolk in a late stage of such modification. Such yolk is met with in two rather different situations: Normally, the whole yolk of the egg (yellow yolk) is subjected during the incubation period to the digestive, *i.e.*, disintegrative action of the embryonic tissues—entoderm and yolk sac. Under such modifying action does yellow yolk become more like white yolk, or does it become less like it? A similar digestive action occasionally overtakes an ovum *in situ*, *i.e.*, while still in the ovary and surrounded by follicular cells. These are the so-called 'resorbed ova.' How does the yolk of such an ovum in an advanced stage of resorption compare with the yellow yolk which it was before the beginning of resorption? Has it become more like, or less like white yolk?

The complete results of my analyses with a consideration of their points of chemical interest, and an account of the preparation of materials, and of methods used, will be published elsewhere.

I may say here that the fat and phosphatid extractions were made with the methods recently discussed and described by Prof. Waldemar Koch, in whose laboratory these analyses have been made. At this time it seems most desirable to present only the amount and sort of data which is necessary to give a clear picture of the major differences between the two forms of yolk under consideration, and to answer the two questions just stated above.

TABLE 2

NO. OF SAMPLE	MOISTURE	IN PER CENT OF SOLIDS			
		Fat	Phosphatids	Extractives	Protein
1.....	47.8	49.2	20.9	0.6	28.8
2.....	63.2	45.7	15.3	2.0	35.2
3.....	49.2	40.7	15.9	2.4	38.7
4.....	88.1	36.8	11.1	3.4	43.5

1 = analysis of fresh egg-yolk (yellow yolk) (17.670 gr.)

2 = analysis of a resorbed ovum (1.834 gr.)

3 = Average of three analyses of contents of (9) yolk sacs (18 da. inc.), (78.821 gr.)

4 = analysis of white yolk (6.019 gr.)

Table 2 has been so arranged as quickly and accurately to tell the story. Nos. 1, 2 and 4 are single and quite typical analyses. The several analyses of the yolk sac contents varied considerably, and therefore an average of three separate analyses of yolk-sacs of eighteen days incubation is here given in preference to a single analysis. The white yolk was taken from a great number of eggs under 6.0 mm. in diameter, the yolk being removed without carrying over any traces of the enveloping membranes.

The quantitative differences in each of these chief components of white and yellow yolk are remarkable. Quite as striking and conclusive, too, are the numbers which show that *when yellow yolk is subjected to digestive action, in either of the two situations named, each and every component approaches more nearly to the quantity characteristic of white yolk.*

It cannot be said, however, that these data conclusively answer the question we have raised as to whether white yolk is an inter-

mediate stage in the formation and indeed of the de-formation (digestion), of yellow yolk; although they do strongly support that view. There seems to be an alternative, namely, that the figures under nos. 2 and 3 approach the composition of white yolk more and more, only because the amount of that sort of yolk originally present in the egg is not diminishing, or is diminishing but slowly, whereas the yellow yolk is here being digested very rapidly. For, it must be remembered that, although we are considering a mature hen's egg as our type of yellow yolk, it still contains white yolk in quantities not easy to estimate; though we are accustomed to think of this amount as small, probably between 5 and 15 per cent of the total.

Parallel to the chemical data are the histological conclusions that it is always white yolk and never yellow yolk that is found applied to a surface into which yolk is being ingested. This is true for the germinal disc of pre-embryonic stages, and for the advancing entoderm and yolk-sac of the embryo (Balfour, Agassiz). Virchow ('91, p. 105) however, questions the correctness of this statement. It is certainly almost always true for the nucleus, or germinal vesicle of the primary oöcyte, a seemingly significant fact upon which I shall publish observations elsewhere. Our chemical data themselves show, however, that the alternative cannot be true unless there is several times as much white yolk in an egg as we have reason to believe exists there. *In any event the certain and interesting fact remains that when the yolk complex of the hen's egg is subjected to digestive and absorptive processes, the fat and phosphatids digest and disappear much more rapidly than does the protein.*

#### ON THE MECHANISM OF YOLK FORMATION AND DE-FORMATION

Having presented data to answer questions three and four of the introductory statement, we may now consider the first and second questions in the light of these results, and with the help of other facts. Precisely how and where does yolk originate? Why or how is it that there are two forms of yolk; or, what is the relation between these?

I purpose to preface this inquiry with a statement of my two main conclusions, or theses. (1.) *The formation and the de-formation of yolk are one and the same subject. The processes of building are also the processes of tearing-down; only an equilibrium changes.* These two sister-subjects have, however, long paraded as independents. The formation of yolk has been considered a subject the investigation of which was connected with a wide variety of study such as the migration of fully-formed yolk granules from follicular cells into the ovum; the origin of yolk granules from migrated particles of the chromatin, or the nucleolus; or again their formation *by* the yolk nucleus, or *by* mitochondria, etc. On the other hand, when the other phase of yolk metabolism—its de-formation—was concerned, observers have been pretty generally satisfied to speak only of ‘a digestion and ingestion of yolk.’

(2.) *Given a region into which the elements of yolk—with their vast amount of potential energy—can go and can exist without undergoing oxidation, and yolk (or some of its elements) will there be increased or decreased in amount subject to an equilibrium which is a function of two factors: (a) the reversible action of enzymes and, (b) the partition coefficient of the elements of yolk.* We do not state that all desirable proof of this thesis is at hand, but we do insist that a very considerable body of evidence supports it. Having been led to the formulation of this view, and to the acceptance of it to the fullest extent ourselves, we shall here outline the evidence which we believe will likewise commend it to others.

It is not necessary to discuss separately what we have called theses one and two. Both rest upon the question of the presence, the effectiveness, and the *modus operandi* of the two factors which we have proposed as the immediate agents of yolk transformations; whether such transformations be of growth or of ‘digestion,’ whether they be progressive or regressive in character. The discussion therefore hangs upon these factors and we shall consider them separately.

Before proceeding in this direction, however, it is well to be reminded that these theses are the physiological and explanatory counterpart of an histological dictum which in certain of its aspects has been for many years ably maintained by several noted

histologists; but which has apparently not gained universal acceptance: *A spherule of yellow yolk may arise from a spherule of white yolk; in the normal destruction and utilisation of the yellow spherule, a white spherule may be again produced.*

1. *The part played by the reversible action of enzymes*

Kastle and Loevenhart ('00) proved the reversibility of the action of lipase—the enzyme concerned in the analysis and synthesis of fat; and we have seen that fat is the chief constituent of yolk. Wohlgemuth ('05) demonstrated the presence of lipase in the yolk of the fowl's egg. It was shown by Henriques and Hansen ('03) that the fatty acids of the food, *i.e.*, of foreign fat, are laid down as such in the hen's egg. Since we know that this fat did not originate within the egg; and, since we are assured that fat as such does not pass through living cells, but that it is previously split into alcohol and constituent fatty acids, we must believe that the foreign fat found by Henriques and Hansen was synthesized *within* the egg cell; or, that it was synthesized in the neighboring follicular cells and thrown from their inner margins into the egg. This last alternative is not true as will be pointed out later.

Thus we come by means of the above series of facts directly to the proof of the existence within the fowl's egg of the *synthesis*—one side of the enzyme action—of the most voluminous constituent of the yolk.

Has the existence of the *splitting* action of lipase in the egg also been demonstrated? I believe it has practically been so demonstrated by Liebermann's ('88) determination that only the merest traces of free fatty acids are present in the fresh egg, whereas large amounts are present at seven and fourteen days of incubation. The existence of a splitting activity of lipase in the hen's egg is moreover a matter that probably no one will question. From these facts then I think it must be said that the reversible action of lipase within the hen's egg has been indirectly demonstrated.

In fact, one familiar with the picture presented by the deposit and absorption of the yolk of eggs, can but wonder that this picture has not been before specifically pointed out as an example—

a typical example—of the reversibility of lipase effecting speedy and rhythmic transformations. The example too, becomes of considerable zoological interest, since certainly nowhere else does this simple physiological principle have such a relation to interesting features of morphology as just here. For, not only does it in these cases often completely change the features of the egg-cell, but it results in a condition (telolecithal) which later gives direction to a host of events of early development—cleavage, gastrulation, etc.—which proceed from the egg.

When we have spoken above of proof of the *synthetic* and of the *analytic* action of lipase we mean, of course, proof that each of these reactions may *predominate in the egg*. The burden of our whole statement is that both sorts of reaction are going on simultaneously (since the reaction is a reversible one); but that the conditions in the egg are, as a matter of observed fact, shown to be such that during the growth period the synthetic reaction normally exceeds the analytic; and that during incubation the reverse is true.

We say nothing in this connection of the origin and disintegration of the proteins of the egg. This group does not furnish, at present, examples specially proved for conditions in the egg, as do the fats. The reversibility of proteolytic enzyme action has however been demonstrated.

With yolk-forming enzymes (lipase, etc.) accelerating a series of reversible reactions in an egg-cell in which traces of yolk have been deposited, what are the factors which favor each side of the reaction, and thus induce either an increase in the amount of yolk, or a decrease in the traces that already exist? We believe that *for the ovum of the fowl* which we shall more specifically consider, some of the factors effective elsewhere may be ignored.<sup>4</sup> The daily temperature fluctuations, for example, are relatively slight, etc. There seems good reason to believe that the *amount and pro-*

<sup>4</sup> There are several other factors or conditions which possibly, even probably, play parts in the *storage of fats*, i. e., building of yolk, in the egg; most of these however are factors supplementary to those of distribution coefficient and enzyme reversibility; though some are not. Some such factors known to be effective in fat-storage elsewhere are: (1) quantity of lipase (Kastle and Loevenhart); (2) different species (?) of lipase (Hanriot); (3) alkalinity

*portion of the reacting substances present* is here, as elsewhere under these conditions, the factor that determines whether the amount of yolk shall from time to time increase, remain constant, or diminish. What then are the conditions in the fowl that would tend to modify the amount of these reacting substances in the egg?

In answer to this we revert to the facts forecasted at the beginning of this paper in regard to our own earlier demonstration ('08) of a daily rhythm of better and poorer nutrition in birds; which rhythms coincide with periods of higher and lower blood-pressure. It was there made certain that very rapidly growing organs (feather-germs) were usually unable to pass over the period of the nightly (1:00-5:00 A.M.) reduction in blood-pressure without showing defects; which defects were proved to be due to insufficient nutrition.

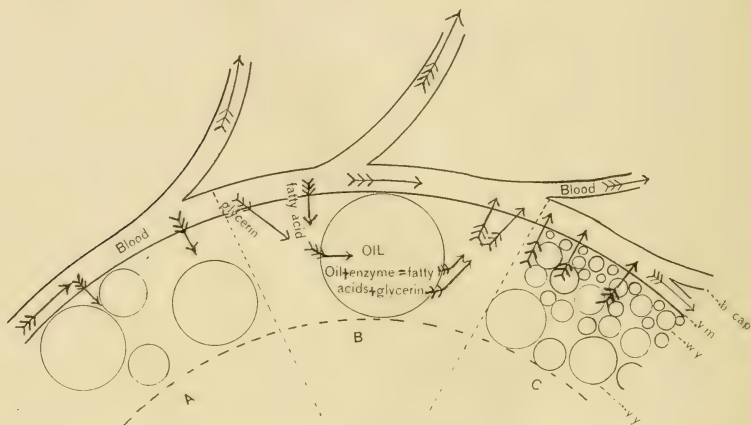
Now we think there is no doubt that these facts lead to an answer to the above question. The egg (like the feather germs) doubtless derives fewer nutritive particles from the blood at this time than during the rest of the day. Possibly, even probably, the low blood-pressure induces at this time feeble but effective currents of fluid from these cells towards the blood and lymph; for it is probable that under low-blood-pressure the volume of the blood tends to increase at the expense of the fluid of the tissues. At any rate it seems certain that at this time the intake of the food substances from the blood is reduced, with the result that the equilibrium of the reaction is shifted. Thus the morphological picture becomes changed. Now growth will proceed more slowly. It is now that the granules must remain small, and poor in fat. It is now that some of the larger yolk spherules (yellow yolk) may possibly suffer reduction to smaller spherules (white yolk);

(Hanriot); (4) presence of other bodies eg. lecithin (Hewlett); (5) reducing conditions, i. e., conditions favorable to the formation of fat from carbohydrate and protein by reduction. A further reason for only a mere mention of these factors here is that the data for the egg are at present too meagre.

The factors which have to do with the formation and storage of the *protein* constituents of yolk, and of their union with lipoids and fats to form yolk, are less known than those factors which involve fat metabolism only; therefore the latter only are treated here. Macallum ('91) has some interesting statements on related subjects, and further points out that *similar processes occur in the formation of yolk and in the production of pancreatic zymogen.*

the former being robbed more rapidly of their fat than of their protein. Now a layer of white yolk is produced in the egg.

In fig. A is shown a diagrammatic representation of how these fluctuations in the quantity of food-products of a fatty nature in the blood-stream would effect changes in size in oil drops, if these latter were separated from the blood by thin and semi-permeable membranes—the conditions existing at the surface of an egg. Section A represents growing conditions—predominance of fat synthesis due to rapid ingress of the constituents of fat. Section B



TEXT FIGURE A=1

Idealized representation of the relations of the periphery of a mature sauropidan egg to the blood and lymph. Follicular cells not shown; these considered pervious as vitelline membrane, or by their intercellular spaces offering free access of lymph to that membrane. A = optimum growth conditions. B = metabolism of an oil drop in equilibrium. C = impoverished blood bearing away elements of yolk, with extension of white yolk area at the expense of yellow yolk. *b. cap.* = blood capillary; *v.m.* = vitelline membrane; *w.y.* = granules of white yolk; *y.y.* = granules of yellow yolk. See text.

stationary conditions; as much of fatty ingredients is being given off into the blood, as is being taken from it. Section C droplets reduced in size as a result of continued contact with a blood stream poor in fat.

## 2. *The rôle of the partition coefficient of the elements of yolk*

Of less importance than the reversible action of enzymes, but following upon it, is the distribution between the yolk<sup>5</sup> and the blood of the soluble substances concerned in these reactions according to their relative solubilities in these two solvents. There can be no doubt that this distribution, or partition coefficient is a factor in determining the amount of soluble substance which comes from the blood, lymph, follicular cells, or vitelline membrane, to the periphery of the yolk, and vice versa. Such is a physical necessity. The constituents of fat for example, quite certainly enter the egg in soluble form and must there be subject to the laws of solubility.

The chief thing incumbent upon us in this connection is to point out how this partition coefficient may act selectively in modifying the amount of the reacting substances in the egg; *i. e.*, how this principle may contrive at one time to increase, and at another to decrease the quantity of yolk contained in the egg. Remembering that it is the *amount* of reacting substances present that decides whether yolk formation or yolk de-formation may occur, the answer can perhaps be more easily given in reference to fig. 1. Let this figure now represent the periphery of the ovum of a turtle, in contact with the lymph and blood streams. During the summer, when the constituents of fat are probably most abundant in the blood, some of these must, on account of their solubility, pass into the egg and there later be built into yolk; their former places being continually taken by new particles from the constant supply of the blood stream. Under these conditions yolk spherules grow, as is represented by section A. In winter, conditions become as in C. The turtle cannot now digest food (Riddle, '09). Its heart-beat and other activities, however, require food for their continuance, and the blood becomes depleted of food. The reversible action of its yolk enzymes is not likewise suppressed, but these now as before set free soluble yolk

<sup>5</sup> The word 'yolk' is here made to stand for the whole body of the egg cell. Perhaps egg-protoplasm, follicular cell, vitelline membrane, and yolk, should all be mentioned instead of 'yolk' alone.

constituents. For such to be set free now, however, is to leave the egg entirely; for now the distribution coefficient of each of such substances brings a portion of it into the blood or lymph; and here it is not allowed to accumulate—to saturate this solution and then cease to act, —but is taken up by other organs; while the blood thus freed from traces of it continues to pick up more of such particles as it passes the ovum. Because of this principle then an ovum may not be able to hold all the yolk that it has once acquired. Apparently we can explain the broad zones of white yolk in the turtles in this way, and the known facts seem to require the mechanisms we have described.

Of course we do not mean to infer that no other factor than the two we are describing have to do with certain aspects of yolk metabolism. For example, these two may have little causative influence in deciding that very important matter as to when the rapid growth of the hen's yolk is to begin. Here lie mysteries perhaps of the follicular cells, or something else, perhaps more distant from the point of actual yolk formation. We are dealing only with the immediate mechanism of yolk formation and deformation.

The possible rôle of lecithin in increasing the solubility of fatty acids and soap in the follicular cells and in the yolk is an attractive subject. Moore and Parker ('01) have shown how enormously the solubilities of these substances are increased by the addition of small amounts of lecithin and bile salts. I have ascertained the presence of lecithin in the follicular membranes, but as yet have not enough analyses for comparison to draw conclusions. I have determined also, as is indicated in Table II, that the lecithin content of the white yolk—*i. e.*, the layer just beneath follicular membrane, and usually between it and the yellow yolk—is smaller in amount than that of the yellow yolk.

As a concluding word on the rôle of the partition coefficient we record our belief that it alone accounts for the presence of the yolk coloring matters—vitello-lutein and vitello-rubin—in the yellow yolk, and not in the white. These are lipochrome pigments, soluble only in fat and fat solvents, and are abundant in the large yolk spherules, probably because, as we have shown by compara-

tive analyses, these spherules abound in fat. Of similar interest is the discovery of Miescher ('97) that at the time of the development of the eggs of the salmon the blood of these animals is unusually rich in lecithin, fat and globulins.

### *3. These two factors and the histological data*

One hardly has a right to mention the words 'histology of yolk' without entering upon the consideration of an enormous literature. Since my own contribution is not primarily of histological nature, and for reasons stated at the outset, I refrain from doing so, although by my results I am seeking to put some rather new and additional interpretations upon histological conclusions, and to answer some questions in which histological, and to a less extent microchemical methods have before been largely used.

The view that intermediate forms of spherules exist, connecting white yolk with the yellow yolk spheres, has been maintained by Rückert, Sarasin, Disse, Kölliker and others. The region under the germinal disc of avian, reptilian and selachian ova have furnished the most and the clearest pictures of the transition forms. Previous authors have, however, generally considered only the formation of the yellow from the white spheres during growth, and have not considered the reverse of this process as it occurs during the destruction of the yolk. The engulfing of whole granules of (white) yolk by the entodermal cells has been recorded by His ('00) and others. This I would observe is, if true, not a real contradiction of my thesis, since these granules doubtless later undergo the ordinary processes of digestion in the entodermal cell. Similarly I would note that the presence of yolk granules in follicular cells—demonstrated by many observers—only illustrates the mechanism we have described at work in another cell; the classic example of this sort of formation being the fat globule in the cell of the intestinal mucosa. On the other hand the finding of such granules in a follicular cell is no guarantee whatever that the granule is thrown as such from that cell into the egg. The granule may here, as in the mucosa cell, again undergo digestion and pass from it in solution.

As regards yolk formation in insects, conditions are peculiar; the nurse cell seems here largely to carry out the work of yolk formation; while certainly the de-formation process normally is carried out by the egg only.

All observers agree that the outermost layer of yolk in any egg or growing oöcyte consists of finely granular yolk. If this were otherwise our general theory of yolk formation would be untenable. Sarasin ('83) was led to the odd idea that the zones of the *Lacerta* egg were developed outermost first, and the central ones last. I think our demonstration of the nature of this growth in the bird, and the considerations that have followed, will convince that Sarasin's view is untenable.

Yolk spherules have been seen to grow after the egg leaves the ovary by Agassiz ('59), Van der Stricht ('07) and others. This growth is quite surely due to the spherule taking up by osmosis water-particles from the albumen or other fluid encountered by the egg; such growth is not of the nature we have described, though neither of the above mentioned authors has made the distinction.

In regard to the conclusion of many cytologists that yolk arises from the egg-nucleus, and of still others that it arises from the follicular cell nuclei, or from these cells *in toto*, I may append the following to show that we can exclude all of these as inadequate in the case of the yellow yolk of the fowl's egg. I have calculated that during the last day that a hen's ovum remains in the ovary it may deposit more than 5000 cubic mm. of yolk! Evidently too much work for an egg-nucleus. Again, since the radius of such an egg is increased by 2.0 mm. per day, this means that if yolk formation be a function of the follicular cell, each such cell must here produce daily a column of yolk 2.0 mm. long and of the diameter of the cell; that is to say each such cell must form more than 50 times its volume of yolk per day, or more than twice its volume per hour! Evidently too much vicarious labor for a cell.

It appears then that an exclusive origin of yolk from the nucleus, or within the follicular cells is impossible in the birds. The quantities of yolk laid down daily are amounts compatible with substances undergoing physical translocation by osmosis, solution,

etc., but not compatible with the probable rate of organic synthesis in the restricted regions of either the nucleus or follicular cell. It is of course necessary for all of the material entering into yolk-formation to pass through or between the follicular cells; but each particle of this material may have, by undergoing the synthesis *in situ* in the egg, twenty-four hours or longer to accomplish this; whereas we have seen that if it originated within the follicle each cell would there have to organize completely its own volume of yolk material and empty itself of this more or less solid material at least once in each twenty or thirty minutes of the day.

Since such theories of yolk formation as have been proposed are now shown to be inadequate in a case where a test can be applied, and since it seems clear that the mechanism of yolk building which we have here outlined and described is necessarily present wherever and whenever yolk is formed, there is at present no valid reason for believing that any dissimilar method of yolk formation exists.

In a certain sense, *no general theory of yolk formation* has as yet been stated. That is to say, no outline of the processes involved in yolk-building and of the conditions affecting these processes has been attempted, and our own effort leaves at least important chemical phases of the problem quite untouched. Previous efforts have been largely devoted to features of the histogenesis of yolk granules, and to the identification of some cell organ as the *directive agent* of yolk formation. Thus such cell structures as centrosome, nucleus, chromatin, nucleolus, mitochondria, yolk-nucleus, etc., have each been several times proposed as the *seat* or *source* of yolk. Whilst for some eggs, particularly those in which all of the yolk plainly could not have so circumscribed an origin, the seat or source of such yolk was centered upon a similar structure of the follicular cell; yolk particles have sometimes been described as arising in such cell and later making their way through the follicle cell membrane, vitelline, or other egg membranes, into the periphery of the egg. But theory usually has extended only to the matter of the source of yolk, to the relation between the white and yellow granules, or to the designation of one or another cell-organ as the directive agent of yolk forma-

tion. There has been no theory to cover the long series of points involved, some of which are the following: What are the conditions which permit yolk to form? In what situations and about what structures does it form (this point much studied and discussed)? What are the processes involved,—what is the mechanism of yolk formation? How are the different forms of yolk genetically and chemically related? How account for the variable amount, distribution, and stratification of yolk?

The statements concerning cell-organs as directive agents of yolk-building have often been quite misleading. This could hardly be otherwise since we have had here attempts to 'explain' a process, not in terms of other processes, but in terms of *structure*—an error not uncommon even in modern biology. One gets the idea from some descriptions of yolk formation that the *nucleus* is the absolute, immediate and ultimate source of yolk; and this in spite of the fact that yolk is never present within the nucleus, but only outside of it. Just *how* a vanishingly small fragment of chromatin, thrust from nucleus into cytoplasm—*i.e.*, into an environment so new as to imperil its own existence,—may guide and direct the very rapid production of a thousand times its own volume of yolk (a new and very different substance from itself) we have not been told. Much apparently has been left to the imagination of the reader who is evidently expected to bridge for himself the gap that exists between the *chromatin particle in situ* and the *yolk building process in operatio*. But, the high regard which some adherents of this theory have for the kingly chromatin evidently persuades them that chromatin particles—which certainly are thrown from the nucleus into the cytoplasm, and about which traces of yolk certainly are sometimes found—comprise material of such superior quality that the base and foreign matter which meets their Midas-like touch must turn at once into golden yolk! By other workers mitochondria, and still other structures, have been similarly endowed with what would seem to be wonderful and transforming power. The writer would not undervalue the great amount of very valuable work that has led to the determination of the cell-elements about which yolk forms. But it seems to him that much less valuable than this painstaking work

are the inferences that have too often accompanied it to the effect that the structure about which *yolk is found to form, is itself the active agent in the yolk formation*. From the facts already brought forward we see that whatever the out-wandering chromatin particle—the invisible *id* or *biophor*—may be able to accomplish in directing the course of differentiation in the highly complex living cytoplasm, the building of a single inert yolk granule by a plainly visible amount of chromatin is a task which clearly quite surpasses it! At any rate a task which it does not accomplish.

Yolk formation as it is indicated by the facts presented in this paper may be connectedly outlined as follows: Yolk will be formed (1) when conditions are such in the egg, follicular cell, food-supply, or organism that excess of food may enter the egg; but (2) in those regions only where some excess of food fat (and protein) can exist without undergoing oxidation; (3) the maintenance of such excess of food is dependent upon the amount of food, or upon marked fluctuations in the amount of food outside the egg, and (4) upon the distribution coefficient of the elements of yolk in the substance inside and immediately outside of the egg, and doubtless by other undetermined conditions within the egg; (5) the actual and active processes of yolk increase or decrease are essentially identified with the partially known synthetic and analytic,—*i.e.*, reversible-action of the enzymes which act upon the constituents of yolk; (6) in the first stages of the growth of a (white yolk) yolk spherule the proportion of fats and phosphatids in its composition is small; (7) in later and more complete (yellow yolk) stages the proportion of these constituents is large.

In my opinion what we now most need to know is how those conditions arise which permit yolk-building to *begin*. We need further knowledge on points (1) and (2) of the above. That is, we need to know why an unusual amount of food enters the egg at this particular time in its history. At present we do not know whether such cause lies inside or outside the egg. Again, what is the source of those *reduction centers* where foods which yield energy so easily as do the fats may not undergo oxidation but be built unchanged into yolk? It is possible, of course that nucleus,

chromatin, mitochondria, or centrosome, etc., of the egg, may later be shown to have special causal significance with regard to such changes in amount of food-intake, or with the production of reducing centers, which we now recognize as basic and unknown features of the conditions which primarily initiate yolk formation. If so, then such cell-structure will have been shown to bear indirect causal relation to a result which it was formerly credited with 'causing' directly; the test of this lies with the future. But, some at least, of the more direct and immediate features of yolk-building are quite certainly those which have been described in these pages.

#### SUMMARY

1. A method of measuring the rate of growth of large, rapidly growing ova has been found. It consists in feeding, at known intervals, the fat stain Sudan III to animals developing such ova.

2. Ova of the common fowl smaller than 6 mm. in diameter grow extremely slowly as compared with ova of larger size.

3. The time interval between the beginning of rapid growth of the 6 mm. egg, and the breaking of the egg from the ovarian follicle (ovulation) is normally between five and eight days. In most cases it is either six or seven days.

4. The radii of ova which are larger than 6 mm. usually increase nearly 2 mm. during each twenty-four hours.

5. The thickness of a layer of white yolk together with an adjacent layer of yellow yolk is nearly 2 mm.

6. A pair of such yolk layers is therefore produced during each twenty-four hours.

7. We conclude that the layer of white yolk in the hen's egg is laid down during poorer nutritive conditions obtaining in the later hours of the night (1-5 A.M.) and that the yellow yolk is deposited during the better growth conditions of the rest of the day.

8. Reasons are found for believing that white yolk wherever found is but a stage in the formation, or the de-formation, of yellow yolk. That it remains as the final form of yolk, only where it is slowly grown or is halted by sub-optimal growth conditions.

Yellow yolk, on the other hand, probably indicates, wherever it is found in ova, rapid growth under better nutritive conditions.

9. The presence of alternating layers or zones of yolk (*Schichtung*) in the ova of some animals thus receives an explanation. A period of poor nutrition corresponds to each of such zones of white yolk; a period of better nutrition to each layer of yellow yolk.

10. The time of formation of a pair of such zones is known in the birds to be one day; in the turtle and myxinoid perhaps a year; in the skate possibly nearly a month; in the lizard this is quite unknown.

11. This '*Schichtung*' of the yolk, and other peculiarities of yolk distribution, have produced great similarity in the gross morphology of eggs of widely separated forms, *e. g.*, selachian and bird; amphibian and marsupial. We can be confident that such similarities do not depend upon heredity in a strict sense, but upon the fact that these eggs have developed under like conditions.

12. The gross chemical composition of white and yellow yolk, and of yellow yolk undergoing de-formation or digestion (*a*) by the embryo and (*b*) by the follicular cells, have been determined and comparisons made.

13. White yolk contains much more water, proteid, and extractives, and much less fat and phosphatid than does yellow yolk.

14. When yellow yolk is digested, in either of the two situations named, its constituent parts are not digested, utilized, and absorbed at a uniform rate; but in such a way that the composition of what remains approaches the gross normal composition of white yolk. In such digestion fat and phosphatid are broken down more rapidly than is protein.

15. The immediate mechanism of yolk formation and of yolk-de-formation are the same. Chiefly involved are two factors—not previously applied here—which we recognize as (*a*) the reversible action of enzymes, and (*b*) the partition coefficients of the several constituents of yolk.

16. The presence of the native lipochrome coloring matter—vitello-lutein—in the large spherules of yellow yolk only, is

probably due to the fact that these spherules contain much fat, and the lipochrome pigment is soluble in fat and fat solvents only.

17. The origin of the yolk of the fowl's egg from the nucleus of this cell, or from the nuclei of the follicular cells, is shown to be impossible. It is not probable that the essential features of yolk synthesis in any egg resides in either of these alleged sources.

18. An attempt is made to outline the processes involved in yolk formation.

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## PLATE 1

### EXPLANATION OF FIGURES

All figures natural size. 1-6 and 1a-6a represent series of eggs grown simultaneously in two Sudan-fed hens. About 20 milligrams of Sudan fed to each hen at 2 P.M., January 27, and at 10 A.M., January 30 (sixty-eight hours).

The bird bearing series 1a-6a killed February 2, 10 A.M. (70 hours after last Sudan began to be deposited in yolk).

1 Egg laid January 27, with pear-shaped, more solid 'waxy' interior; also two prominent circles of 'modified yolk' near periphery.

2 Egg laid January 29. The outer border line here represents Sudan deposited from feeding of January 27. This layer was 1 mm. in thickness. The two circles of 'modified yolk' showing here as in figs. 1, 3 and 4. The size of each of the yolks at the time of the modification is indicated by these circles.

3 Egg laid January 31; see above.

4 Egg laid February 2. Two layers of Sudan. The time between Sudan feedings was sixty-eight hours; the amount of yolk deposited in this egg during that time was 6.2 mm. = 2.2 mm. in twenty-four hours. Section nearly in plane of germ.

5 Egg laid February 4. The two layers of Sudan here as in fig. 4, were 6.2 mm. apart = growth of 2.2 mm. in twenty-four hours. Section at right angles to plane of germ.

6 Egg laid February 7. Shows spreading, or dilation, effects in Sudan layers. Apparently the 'spreading' is mostly outwards, though this figure well represents neither the position nor the condition of each layer. This effect noted in eggs that have remained long in ovary, or, as in this case, in laboratory at high temperatures.

1a Egg laid January 27. To unaided eye the outer 10 mm. of one side of this egg showed very plainly six pairs of yolk layers = 1.67 mm. each. Interior contained somewhat solid, waxy body  $15 \times 10$  mm.

2a Egg laid January 29. Seven very distinct pairs of layers of white and yellow yolk. The white yolk represented by dotted lines; the yellow yolk by the spaces between these. Probably another layer central to those indicated in figure.

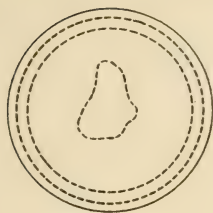
3a Egg  $40 \times 27 \times 28$  taken from oviduct (see first statement above). Inner borders of two Sudan layers are 4 mm. apart = 1.41 mm. growth per twenty-four hours. Section through plane of germ.

4a Egg  $31 \times 26 \times 28$  from ovary (February 2). Distance between inner borders of Sudan lines = 3.6 mm. = 1.3 mm. in twenty-four hours. Section nearly in plane of germ. Figs. 3a and 4a show the spreading or diffusion of Sudan in the region of the germ. Distance between inner border of outer Sudan layer and periphery = 5 mm.; this growth of seventy hours = growth of 1.7 mm. in twenty-four hours.

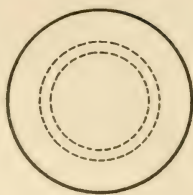
5a Egg  $24 \times 21 \times 20$ , from ovary (February 2). The first feeding of Sudan (January 27) left but faint traces of the dye in this small egg (see small crescent). By mistake the inner border of the thick layer of Sudan of this figure was placed 7 mm. from periphery instead of 5 as it actually was. This 5 mm. = growth of seventy hours = 1.7 mm. growth in twenty-four hours.

6a Egg  $15 \times 16 \times 19$ , from ovary (February 2). Smallest egg in which trace of Sudan was present; this somewhat diffuse and indicated by dotted circle. From inner border of circle to periphery = 4.8 mm. the growth of seventy hours = 1.64 mm. growth in twenty-four hours.

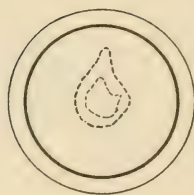
All eggs are drawn as perfect circles, although, as is indicated above, the boiled yolks quite constantly show three unequal diameters.



1



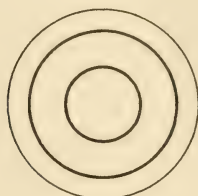
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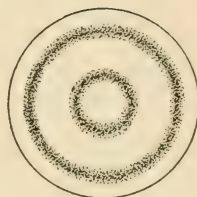
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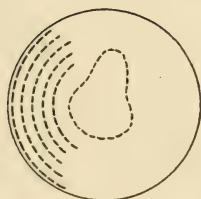
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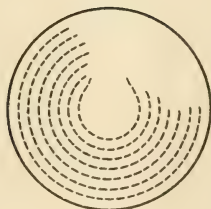
5



6



1a



2a



3a



4a



5a



6a

## PLATE 2

### EXPLANATION OF FIGURES

Figures all drawn twice natural size, and then reduced one-sixth.

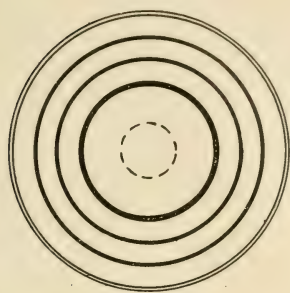
1 Section at right angles to germ of an egg ( $32 \times 30 \times 28$ ) that showed with schematic clearness its rate of growth. The Sudan deposited in the first (inner) broken line was fed forty-eight hours before a following feeding. Thereafter the feedings of the dye were made at thirty-six hour intervals. A growth of 1.8 mm. per twenty-four hours is indicated for the three intervals of thirty-six hours.

2 Through germ of egg,  $33 \times 29 \times 27$ . Shows well the manner of deposit of dye (and therefore of yolk) in immediate region of germ. The fan-shaped figure of dilute Sudan lying deeply beneath the germ is perhaps however not a correct picture of the original position of the dye, but a 'spreading' effect. The first two feedings of Sudan were here twenty-four hours apart; the next forty-eight hours and the last, twenty-four hours later. A growth of 1.5 mm. per twenty-four hours is here indicated for time between first and last feedings.

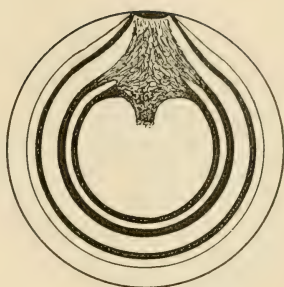
3 A series of small ova (4-7.5 mm. in diameter) from the ovary of a laying hen which had been once fed about 25 milligrams of Sudan and killed on following day. Only three of these ova (6.5 to 7.5 mm.) showed any trace of the dye. In the drawing of the egg of 7.5 mm. the position of the layer of dye was placed two mm. too near center of egg. The number along side each ovum indicates its actual diameter in millimeters. The egg of 7 mm. was of special interest. After lying in a quantity of Mann's balanced formalin-alcohol solution for a few weeks the striated appearance of the outer portion of its *white* yolk was visible with binocular. The lower right hand figure is an attempt to represent the structure of the outer 1.75 mm. of the 7 mm. egg. Eight striæ could be here distinguished. Apparently therefore the striæ have a thickness of about 0.25 mm. The stain was found to be confined to the large, yellow yolk granules.

4 Egg,  $34 \times 29 \times 31$ , through plane of germ. Central heavy irregular lines are from Sudan feeding three days apart. Bird was laying at long intervals and next feeding delayed ten days and following this two feedings two days apart. This egg therefore at least seventeen days in developing. But its abnormality is evidenced by the crumpled appearance of the innermost layers of Sudan (the corners of innermost layer are too sharp in drawing) and by a curious depression of the germ. Another peculiarity of this egg was its presentation of a brightly Sudan-colored vegetative pole (the stratum of dye here near surface), and a normally colored animal pole (except for the small depressed region of germ).

5 Later egg, sister to no. 4. Much more rapid growth than in 4. First two feedings thirty-six hours apart; others twenty-four. A growth of 1.3 mm. per twenty-four hours is here indicated.



1



2



4



5



6



$6\frac{1}{2}$



$7\frac{1}{2}$



$5\frac{3}{4}$



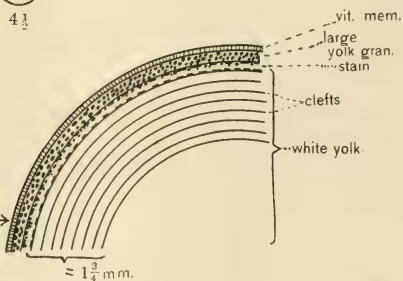
$4\frac{1}{2}$



$6\frac{1}{4}$



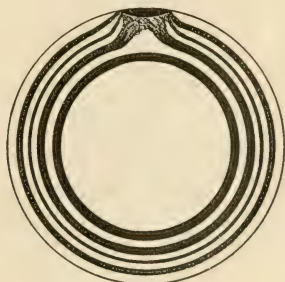
7 mm.



3



4



5

## PLATE 3

### EXPLANATION OF FIGURES

The distribution of white and yellow yolk in the ova of vertebrata with special reference to zonal or lamellar formation.

1 Egg of tortoise, *Clemmys* (from Munson, '04), *c.c.* = cytotcenter (centrosphere); *y.c.* = inner cytoceol of Munson, and what I should call inner or first layer of white yolk; *i.y.l.* = inner yolk layer of Munson, and inner or first yellow yolk layer I have called it; *o.cy.c* = outer cytoceol or second layer of white yolk; *o. y. l.* = outer yolk layer, or second layer of yellow yolk; *s. c. l.* = subcuticular layer.

2 Mature egg of *Petromyzon* (from Herfort, '00). Very small granules in the external oöplasm, gradually merging into the large granules and large vacuoles of internal oöplasm.

3 Nearly grown egg of *Phascolaretus* (marsupial) (from Caldwell, '87). The darker crescentic body is coarsely granular yellow yolk; the clear area around the nucleus, which is also continued around the periphery of the entire egg is of finely granular white yolk.

4 One end of large (3 cm. long) egg of *Bdellostoma* (cyclostome) to show stratification of its yolk (from Dean, '99). The fine curved lines represent points richest in minute yolk-spherules (white yolk).

5 Mature egg of *Ichthyophis glutinosa* (amphibia) (from the Sarasins, '87)  $6 \times 9$  mm. with central 'latebra' of white yolk; this connects above with the germinal vesicle, forming a nucleus of Pander beneath the latter.

6 Mature egg of *Torpedo* (from Rückert '99) in meridional section. The lens-like germ above. A central 'latebra' without stratification (Rückert says this is composed of dark, not light, substance). The dark layers are composed of loosely bound, but larger yolk platelets (white yolk?); the wider lighter strata of more closely packed but somewhat larger platelets (yellow yolk?).

7 Hen's egg photographed to show something of the concentric deposition of Sudan III. Dark lines = Sudan; these bright orange-red in original. The appearance here is very similar to the always less evident stratification of white and yellow yolk; the narrow lines of Sudan in the photograph simulating the faint and narrow lines of the white yolk.

8 Part of immature egg of *Lacerta* (from Sarasin, '83) showing well-marked layers of white and yellow yolk (I infer that the dark lines represent white yolk); about one-fourth of egg is here shown. The germinal vesicle lies just outside the figure, above and to the right; all layers are seen to converge toward it, and to become gradually modified in its vicinity.



1



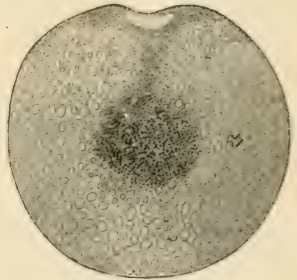
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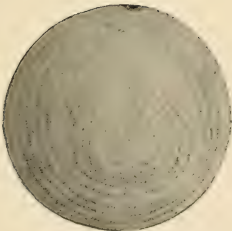
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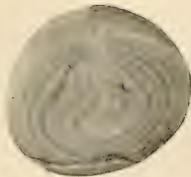
4



5



6



7



8



# SOME PROBLEMS OF COELENTERATE ONTOGENY

CHARLES W. HARGITT

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THREE PLATES AND THREE TEXT FIGURES

## CONTENTS

Introduction.....	494
Material and methods.....	495
Observations.....	497
A Pennaria.....	497
1 Cleavage.....	499
2 Nuclear aspects.....	499
3 Amitosis.....	501
B Hydraetinia echinata Flem.....	502
1 Cleavage.....	503
2 Ectosareal features.....	508
3 Early embryo, morula.....	508
4 Organization of the embryo.....	509
5 Entoderm formation.....	509
6 The larva, planula.....	510
C Clava leptostyla Ag.....	511
1 Maturation.....	511
2 Nuclear behavior.....	512
3 The chromatin.....	513
4 Nucleolar behavior.....	516
5 Later development.....	517
6 The morula.....	518
7 The germ layers.....	518
a. Ectoderm.....	519
b. Entoderm.....	520
Review and discussion.....	523
1 Origin and growth of germ-cells.....	524
2 Doctrines of homology.....	529
a The germ layers.....	531
b The planula.....	531
c The morula.....	532
d The blastocoel.....	534
e Cleavage homology.....	535
3 Amitosis.....	537
Summary.....	541
Bibliography.....	542

## INTRODUCTION

In the course of investigations carried on by the writer during several years, certain facts have come to light which seem to have important bearings upon several problems of general ontogeny. In various papers phases of these have been suggested, but only incidentally has any attempt been made to discuss their significance or their probable correlations as developmental phenomena. With further investigations still additional facts have been observed, and similar investigations by others have tended to convince me of their importance in a still larger degree. When the honor to coöperate in the preparation of this memorial volume was submitted, it seemed that no more appropriate subject came within the scope of the writer's researches than that involved or implied in the above caption.

My introduction to coelenterate morphology began many years ago with the problem of the origin of sex-cells, a subject at that time brilliantly exploited by Weismann, whose "*Entstehung der Sexualzellen bei den Hydromedusen, Zugleich ein Beitrag zur Kenntniss des Baues und der Lebenserscheinungen*" ('83), has long been a recognized classic in its line. It was ably supplemented by the hardly less brilliant researches of Metschnikoff ('86), "*Embryologische Studien an Medusen. Ein Beitrag zur Genealogie der primitiv Organe.*"

The first contribution to the subject by the writer was a very brief and tentative paper before Section F, of the American Association for the Advancement of Science, in 1889. It was adversely commented upon by one who had accepted without question the then prevalent dogma that Hydrozoa were distinguished from all other Cnidaria by the origin of the sex-cells exclusively from the ectoderm. Under this adverse criticism no further utterance was made on the subject for several years, though there was no lapse of interest or investigation.

In the meantime, an observer here and there had dared to question the conclusiveness of the earlier dogma. Little by little facts were accumulating which cast further doubts upon the matter, and even compelled the conclusion that Weismann's

fundamental contention was inconclusive. Results to be cited from various sources will tend to show that the early attempt to formulate a general theory of embryogeny on the basis of the origin of sex-cells was no less defective and inadequate than it was hasty.

For some time past phases of my researches have forced the impression, which has deepened as the investigations have extended, that not a few of the earlier views as to coelenterate ontogeny were seriously defective, or absolutely in error at many points. Certain of these I have taken occasion to point out from time to time, as occasion arose. The purpose of the present paper is two-fold: First, to submit accounts of the development of several species of Hydromedusae which have been under investigation for some time; and secondly, to point out certain errors as to the ontogeny of the groups which, from various reasons, had become associated therewith.

#### MATERIAL AND METHODS

1. *Material.* The material upon which the results herein described are based (with the exception of that of *Pennaria australis*, for which I am indebted to Mr. Edgar J. Bradley, of Australia, to whom my thanks are hereby acknowledged) was collected by the writer at various times within the past two years, and chiefly in the immediate vicinity of Woods Hole, though some of that of *Clava* was collected at Harpswell, Maine. It is a pleasure to express my thanks to the directors of these laboratories for various courtesies.

Attention will be given primarily to two species of *Pennaria*, and to a single species each of *Clava* and *Hydractinia*. Other species will be given attention in relation to the several problems with which the paper has to do.

2. *Fixation.* In my earlier work great difficulty was encountered in reference to killing and fixing reagents. For killing my first lots of eggs of *Pennaria* picro-nitric and picro-sulphuric solutions, then much in vogue, were used; but to my sorrow these were found to be almost worthless. This was particularly the case with picro-sulphuric. Almost the whole of one summer's

collection was absolutely worthless by reason of the almost exclusive use of this reagent.

Hermann's and Flemming's solutions afforded fairly good fixation, but subsequent staining was very difficult. Perenyi's solution was absolutely worthless with both Eudendrium and Pennaria material and has since been discarded. The only solution which gave reasonably good and fairly constant results was a strong solution of corrosive sublimate to which had been added 5 per cent of glacial acetic acid.

In later work I made use of various solutions of formaldehyde, but with only fair results. A 10 per cent solution in sea-water gave a good general fixation for immediate use. Combination with corrosive did not seem materially to better it. There was found also to be great variability in different species as to this matter. This was particularly apparent in eggs heavily yolk laden as compared with those in which yolk was lacking, or present in only small quantities. There was also great difference in later differentiating other cytoplasmic elements. For example, in the peculiar proteid granules present in eggs of *Clava* the first, and only satisfactory reagent was picro-acetic acid (p. 217, Biol. Bull., vol. 10, '06).

In 1906 my attention was directed to Bouin's picro-acetic-formol. It was thoroughly tested upon eggs of *Pennaria* and *Hydractinia*, and was found to be far superior to any thus far employed. I have since used Zenker's fluid with good results in fixation of eggs of several species. It is worth while to emphasize the importance of this feature of fixation, especially as it relates to coelenterate material. I have called attention to this in several previous papers, but it is absolutely imperative in order to warrant trustworthy results that particular attention be given to this matter.

3. *Imbedding.* In another respect I have learned to my cost the importance of prompt working up of coelenterate material after fixation. Attention was directed to this point in my paper on *Pennaria* ('04b, p. 455). This precaution has been abundantly confirmed by later experience, and I take occasion here to emphasize its importance once more. The value of this has been

vouched for by Smallwood ('09). My present method in this particular is to imbed the material in paraffine as early as possible after reasonable time has been given for proper hardening and dehydration. This imbedded preservation may apparently be indefinitely prolonged without detriment. But in my experience it is impossible to preserve material of this group for any considerable period in alcohol without having it suffer considerable deterioration. This is particularly the case with those cytologic factors of mitosis and allied features so important in modern problems of embryology.

4. *Staining.* This, like the matter of killing and preservation is one of much importance and of varying grades of difficulty, as it related to the problem under review. As in the preceding, I had long since called attention to the extreme difficulty in the staining reactions of coelenterate material. This was most marked, in my experience, in the eggs of Eudendrium and Pennaria. Others have also found similar difficulties with this phase of technique. G. T. Hargitt ('09, p. 163) has recently devoted some attention to the subject, and my own results have been confirmed by those described in his paper.

Difficulties experienced in my earlier work in Pennaria, and the later work on Clava, were such as to leave doubt, particularly in relation to the phenomena of maturation, leading me to conclusions, tentatively adduced, which subsequent work has not confirmed, as shown by G. T. Hargitt (op. cit.) and Smallwood ('09), and by facts herein described.

## OBSERVATIONS

### A. *Pennaria*

Except for additional facts which have come to light in relation to a species of Pennaria, the development of which has been hitherto unknown, no particular attention would be given to the subject in this connection. Since the issue of my detailed paper on the early development of Pennaria tiarella ('04), repeated observations on the living eggs have confirmed my previous

results in every detail, so far as the general facts are concerned. I think it may now be regarded as beyond doubt or cavil that these results, anomalous as they may appear, are absolutely normal and conclusive. Furthermore, when analogous cases to which I had directed attention, and others to be cited in a later connection, are taken into consideration, it seems rather strange that "early cleavage differing widely from what we have come to think as typical" should be given as adequate grounds for a reëxamination of the case! However, when it is recalled that, with certain investigators, it is more important to reduce vital phenomena to a set of formulae, or to corral all development within a common law than to recognize facts as they are, the wonder is less strange than it might at first seem! But additional facts are now available from a most unexpected source, and of such character as to remove any further grounds for question or doubt.

Somewhat over a year ago I had the good fortune to receive from Mr. Edgar J. Bradley, of Adelaide, Australia, a collection of hydroids, and along with them several colonies of *Pennaria australis* Bale, together with the medusæ and eggs, which had been taken in tow-nets just at the height of the breeding season. The only feature of regret as to the eggs is that they had not been preserved in other than weak formalin, in order to have made them available for cytological study. But, as it is, they show in surface study the external aspects of developmental behavior to such perfection as to leave little to be desired. Figures 5 to 8 are sketches of a few of these stages, which speak for themselves. As will be seen at a glance, they duplicate in a most striking way similar stages in the development of *Pennaria tiarella*. If one were to pass under review separate series of eggs of the two species, without pains to have critically determined them in advance, it would be practically impossible to say which belonged to the one species and which to the other. There are the same ectosarcal features,—papillæ, bridges, strands, etc., in both; the same bizarre, amoeboid characters, the same anomalous phases of cleavage, 'every egg a law unto itself', and finally the same end resultant, a normal embryo. Later phases of development of the Australian species were not present, hence further compari-

son was impossible, though there is no reason for doubt as to its subsequent similitude and results. A comparison with figures 1 to 4, of *Pennaria tiarella*, will make this more evident.

The fact that these eggs had been taken with the tow-net in the open harbor, and had been preserved shortly after in formalin, leaves no grounds for serious question as to their normal condition, and confirms completely the results of my own precautions ('04b, p. 474), to guard against possible effects of artificial conditions of the laboratory. These additional facts, together with others of like character which have since come to our knowledge, especially those described by Brooks and Rittenhouse ('07) must suffice once for all to establish the perfectly natural and normal phenomena of extremely erratic and indeterminate modes of cleavage and consequent organogeny.

1. *Cleavage*. There is nothing new to add concerning the cleavage features of the eggs of *Pennaria tiarella*. Concerning this feature in *Pennaria australis* little attempt will be made to give detailed descriptions. The figures cited will afford all that is necessary as to the general surface aspects. As already stated, there is such essential conformity in every respect to the corresponding stages in *Pennaria tiarella* that there seems small occasion to do more than refer to the figures and descriptions of the former paper ('04b). While the fixation does not give material fit for cytologic details, it is fairly good for general comparisons. Eggs carefully stained and cleared show fairly well the general internal conditions, and here, as in the surface features, there is essential likeness to corresponding stages in *Pennaria tiarella*.

2. *Nuclear aspects*. Brief reference may be made to a few points under this head.

*Fragmentation*. In several of my earlier papers ('04b, pp. 460-1), attention was called to certain nuclear phenomena of a rather peculiar character. Among these was what seems to be a rather promiscuous dissolution, or disintegration of the nucleus and the dispersion of the greater portion of it into the cytoplasm. To designate this process I used the term fragmentation, long previously employed to designate phases associated with direct nuclear division, and apparently first employed by Van Beneden (Wilson, the Cell, p. 64).

In recent papers both Smallwood ('09) and G. T. Hargitt ('09, pp. 197-8), have expressed doubt as to the process in the eggs of *Pennaria*, the latter stating that "no sign of its fragmentation has ever been seen." But in the following sentence he adds, "the supposed disappearance of the germinative vesicle at this time, I believe to be due simply to the usual dissolution of the nuclear membrane and the mingling of karyoplasm with cytoplasm."

Smallwood expresses similar doubt, saying:

If by fragmentation of the nucleus is meant that the entire nucleus disappears and its contents disperse throughout the cytoplasm, then I find no evidence of such a process in these hydroids. But what shall be said of the chromatin changes before maturation in *Hydractinia* and in *Pennaria* after maturation, where large quantities of chromatin migrate into the cytoplasm? (Op. cit., p. 228.)

It was chiefly in this latter sense that I had used the term, and observations of Coe, Lillie, and others were cited in support of facts found in *Pennaria*. It may also be admitted that there seemed to be evidences of the *entire dispersal* of nuclear substance through the cytoplasm and their subsequent reorganization into new nuclei. ('06, p. 227, etc.). Further reference to this will be made in another section.

Contention for fragmentation was based almost wholly on chromatin behavior. The facts which I urged in this connection were those involving, *first*, the enormous dissipation of chromatin and its absorption by the cytoplasm, during the phases of maturation; and *secondly*, the achromophilous condition of the chromatin at a slightly earlier time. *These facts have not been disputed.* Whether my inferences or interpretations are valid is quite another matter. As to that upon which I have laid most stress, viz., the disintegration and dispersal of a preponderating portion of the chromatin, certainly not less than 90 per cent in many cases, and that it has little or no subsequent function as chromatin, —I am still firmly convinced of its validity and of the vast significance it involves as to chromosome theory.

Concerning the achromophilous condition above referred to, I have little to add to my previous accounts. G. T. Hargitt

('09, p. 165), whose detailed experiments on differential staining have surpassed my own, was perplexed as to this condition. "At the end of the growth period, the nuclear reticulum shows so little affinity for basic stains that there appears to be, so far as this test shows, no chromatin present in the entire nucleus. I can suggest no explanation for this peculiar condition of the chromatin at this period, but it is normal and characteristic of this stage." I am now convinced that there is here a chief ground for my failure to distinguish certain phases of maturation, and my subsequent error in the assumption of their possible suppression or modification in certain cases.

3. *Amitosis*. Concerning a further problem, that of amitosis, I am in doubt so far as *Pennaria* is concerned, even as at the time of my previous work. My chief grounds for this view are the facts first cited, and those of the multivesiculate aspects of the nucleus during cell proliferation. And here again Smallwood and G. T. Hargitt ('09), and later Beckwith ('09), all confirm my basis of facts. They find in these vesiculate nuclear conditions essentially the same results which are described in my accounts. Without exception their interpretations differ from mine. To them these facts are believed to be obscure phases, chiefly telophases, of mitosis. While I freely admit the force of their contentions, there are still good reasons for maintaining the plausibility of my own views and interpretations. This is especially the case concerning *Eudendrium*. Here there seemed to be clear and positive examples of amitosis, as shown in fig. 23, *a* and *b*, plate 15 ('04a). It may not be amiss to state here that all these examples of amitosis occurred in association with those 'nuclear nests' so intimately involved in the syncytial phase of development concerned in entoderm formation. The conditions are somewhat different in *Pennaria*, yet sufficiently similar to lead one to anticipate similar processes, and these appeared probable in the vesiculate 'nuclear nests' mentioned above. But in no case were there found the specific and positive examples figured in the case of *Eudendrium*. The same must also be said of *Clava*. But further discussion of this will be reserved for a later section.

*B. Hydractinia echinata Flem.*

During the summer of 1907 I was fortunate in securing large numbers of this hydroid in the height of its breeding season, and took occasion to study the development and life history of the species. Some account of the life history has already been given ('08) which obviates any call for emphasis here on this point. The early development was studied from living material during two summers and at the same time material was carefully preserved for cytological study. This latter was turned over to my colleague, Dr. Smallwood, and his results have already appeared ('09). It only remains for me to submit such accounts of my observations as seem important in order to afford a more or less complete and connected description of phases of development, especially when correlated with Smallwood's account referred to above.

There are numerous points of difference between my observations and those of Bunting ('94), some of which may be due to the fact that her studies were restricted to material obtained from the small colonies living upon shells occupied by hermit crabs, while my material was derived chiefly from colonies of enormous size, obtained from piles of docks or similar habitat, but with comparison from the former sorts. As pointed out in the paper referred to above ('08, p. 98), there is no adequate reason for regarding these hydroids as other than a single species, hence any differences to be cited must be incidental rather than fundamental.

One of the first points of difference to be noted is concerning the time at which the liberation of sexual products takes place. According to Bunting this is between the hours of 9:30 and 10:30 P. M. That it occurs during the night I have repeatedly demonstrated. Further, that it may occur in certain cases *about* the time stated by Miss Bunting, I have also found true. But that it may also occur at a much later hour, and also at varying hours, I have also found to be the case. Some of the best cleavage series obtained, especially for the very early stages, were in the mornings from seven to nine o'clock. That is to say, the eggs had been deposited some time after midnight, and at the hours

named were in early stages (two- to eight-cell) of cleavage. This would seem strongly to indicate their deposition at perhaps five or six o'clock in the morning or thereabout, as recorded in my notes of July 11th and 12th. In other cases development had reached the morula stage at nine A. M., which would lead to the conclusion that liberation of sexual products had occurred about midnight. While it is true that in many hydroids the liberation of eggs and sperm occurs at a fairly constant time, yet there are others in which this is not the case, and in which such ripening and discharge is a more or less continuous process during the breeding period.

The character of the egg is much like that of *Pennaria*, though it is much smaller. Both are alike in general texture of protoplasm, contain yolk, and similar inclusions. There is present a pigment similar to that in the eggs of *Pennaria*, though less marked in color. Like those of the latter, the eggs are devoid of a definite membrane. They are rather heavy and sink promptly when set free. By reason of this it was practicable to suspend colonies in shallow vessels within wire baskets under docks in freely circulating water and with little liability of their being lost. This was a matter of some importance; for, despite the best precautions, these hydroids soon deteriorate in vitality under the artificial conditions of the laboratory, while by suspending them in open waters about the docks they thrive almost as if in the natural habitat.

1. *Cleavage.* So far as I am aware the only definite work on cleavage of *Hydractinia* is that of Bunting ('94). In this paper we have a characteristically symmetrical portrayal of the process. In general surface aspects it is represented as almost mathematical in its regularity and symmetry.

That the earlier cleavage phases in perhaps a majority of the eggs conform to this in greater or less degree is probably true. But that it represents with any degree of accuracy the average behavior of this phenomenon as a whole none who had carefully followed it could for a moment admit. It has been difficult to conceive how, except by a *selective process*, any such account could have been formulated. It is quite easy to see that by directing

attention only to eggs which exhibited the regulation aspects of cleavage, and disregarding, as abnormal, those of differing aspects, just such an account might easily have been made up; and this in all probability may have been the method followed.

It is not strange that under prevailing conceptions as to formulated 'laws of cleavage' this method might naturally have been adopted. In the case of *Pennaria* the present writer deliberately disregarded an entire batch of eggs which were so erratic in behavior as to suggest the probability of pathological conditions. But, by whatever method one may explain the matter, certain it is that there is a measure of irregularity in a large proportion of the eggs of *Hydractinia*, especially after the third or fourth cleavage furrows, which at once takes them out of the usual category of geometrical order or symmetry and puts them, if not in the *Pennaria* class of chaotic irregularity, at least consigns them to the category of the indeterminate and unsymmetrical.

However, it is not my purpose, in thus discrediting an account which gives so inadequate and misleading an impression, to go to a similar extreme in the other direction and convey the impression of predominantly erratic cleavage. On the contrary, let it be noted that in perhaps a majority of the eggs of *Hydractinia echinata* the cleavage, while seldom exhibiting an approach to geometric order or symmetry, is yet more or less regular and orderly. In such cases cleavage begins, as usual, at the animal pole, cutting vertically downward, and generally divides the egg into symmetrical halves, which adhere to each other by a narrow band, or connective of cytoplasm at the lower pole. The second cleavage likewise may begin at the upper pole and at right angles to the previous division, or may begin at the center and work outward, thus dividing each half into symmetrical fourths, giving a fairly typical four-cell stage. The third cleavage, which is usually equatorial, often begins at the center and extends toward the periphery, a process more or less common in eggs of hydroids. The subsequent phases may continue more or less orderly as in earlier stages, but often grow increasingly irregular and independent, though resulting in a symmetrical embryo. On the other hand, figs. 14 to 22, which are camera sketches of living eggs,

show how strikingly irregular and unsymmetrical cleavage may be in eggs of a given lot, developing under identical conditions. But in these cases the first two or three cleavage furrows are more or less symmetrical. In not a few, however, cleavage is distinctly erratic from the first, the first furrow dividing the egg into very unequal portions. In such cases the irregularity becomes usually increasingly more so as cleavage goes forward.

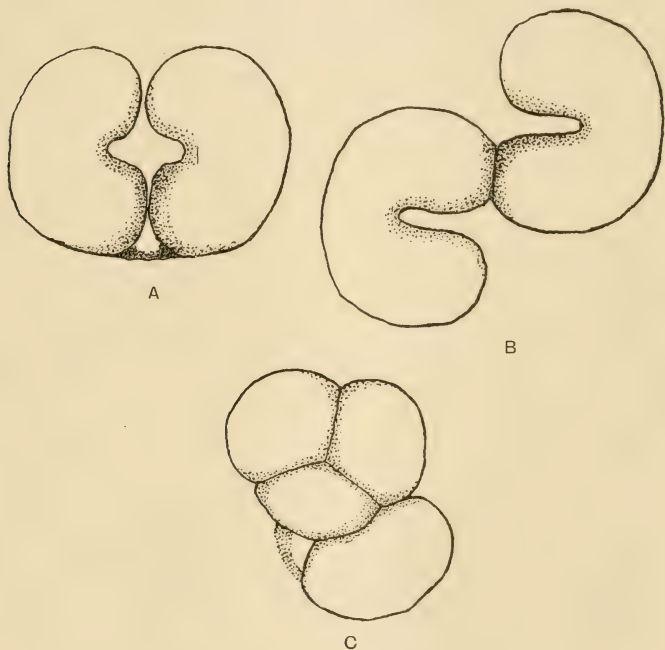
A very interesting case is that shown in figs. 9 to 13, which occurred at irregular intervals within a period of about forty minutes of constant observation during which the sketches were made. The egg was kept under observation for several hours, or till the morula was apparently completely formed. Fig. 9 shows what may be regarded as a four-cell stage, the central portion comprising the main body of the cell, while at opposite poles are three other blastomeres, in each of which the nuclei were distinctly visible. In fig. 10 the small blastomere at the upper pole has divided, so that now we have a five-cell stage. It remained in this condition about fifteen minutes, when a most curious thing happened, the small blastomeres, *x* and *y*, being the factors of most interest. At first the blastomere *y* became detached from its connection with the cell body as shown in the figure, and later the other blastomere *x* did the same thing, both thus becoming absolutely free, in which condition they continued about thirty minutes. At the end of this time they resumed division and went forward to complete segmentation and formed what seemed to be a perfect, though very small morula, shown in fig. 11, *b*. The other portion exhibited something of the same tendency. For example, the small blastomere *z* cut itself free as had *y*, but it later drew back and, fusing with the cell body, continued as an integral part of the egg in its later development. Figs. 12 and 13 show the general aspects of this portion, which went forward normally and became a perfect morula and later gave origin to a normal planula. The small segment exhibited the same aspects, but later in the day began to show signs of degenerative tendency and finally disintegrated entirely without assuming the larval condition. This can hardly be ascribed to its minute size, for other embryos, which were otherwise apparently similar in every way, suffered the same fate.

Among the anomalous aspects of cleavage which I have encountered in the development of these and other hydroid eggs not the least singular or significant is the occurrence, now and then, of what may be designated as blastomeric autotomy. That is to say, occasionally one finds during the earlier stages of cleavage, most commonly at the first, the complete separation of the primary blastomeres, which continue to develop as independent eggs, and from which independent embryos arise, giving origin to two polyps. I have called attention to something of this in *Pennaria*. The same thing has been found in at least two cases in *Hydractinia*. In one case actually followed from beginning to end the sequence of events may be briefly described. At the first cleavage of an egg which was in a marked degree unequal, the two blastomeres separated entirely, each part developing quite apart, and in a perfectly independent fashion. One of these segmented in a fairly regular and symmetrical fashion, while the other portion was markedly irregular from the first. It should be observed that the rate of cleavage in the former was much slower than in the latter, which exhibited a marked tendency toward amoeboid aspects as shown in the figures already cited.

It seems perfectly clear, therefore, that we have in these aspects of development a perfectly normal, and not particularly rare mode of segmentation, involving the origin of two, and probably even three or more embryos from a single egg, in a perfectly natural and spontaneous way.

Among these anomalous aspects, which were numerous as well as various, those shown in text figs. *A*, *B* and *C* will be interesting. In this case the first cleavage was about normal, beginning at the animal pole and extending downward to the lower, where the blastomeres remained attached by the connective shown in fig. *A* for some time. The second cleavage was the not unusual type shown in fig. *B* where it was directed centrifugally and in a horizontal instead of a vertical plane; and as it continued the connective was resorbed, leaving the two blastomeres quite free for a time during which they moved into the position shown in the figure, when the vegetal blastomere of the one side came into contact with the animal blastomere of the opposite part, in which posi-

tion they fused and remained for some time. Fig. *C* shows the condition when the four-cell stage had been established. As will be seen, the blastomeres had rotated until they became, as it were, fitted into close contact with each other, and the development of another connective bound them in that relation for some time. It may be noted that later development went forward with average regularity.



2. *Ectosarcal features.* In my paper on *Pennaria* ('04b, p. 469) attention was directed to certain very conspicuous aspects which were designated as 'ectosarcal phenomena,' and which comprised various more or less superficial excrescences, such as papillae, films or bridge-like connectives between blastomeres,

etc. They were described in some detail and various suggestions and comparisons submitted as to their significance.

In the eggs of *Hydractinia* very similar structures were encountered, though less conspicuous and less constant than in *Pennaria*. Certain of these are shown in figs. 14 to 22. As to their significance or function I have nothing new to offer beyond that previously suggested. Their more obvious function would seem the two-fold one of connecting adjacent blastomeres, and affording coördinating bonds for the entire egg during development. These suggestions could hardly apply to the papillose structures of the surface, and their presence must be regarded as problematical.

3. *The early embryo, morula.* With the progress of cleavage toward completion the irregularities of surface, due to ectosareal structures above mentioned and erratic cleavage, which were so conspicuous a feature, gradually disappear and the embryo tends to become more or less typically spherical and blastula-like. But in comparatively few *Hydrozoa* does a typical blastula occur. In my earlier accounts of the development of *Eudendrium* and *Pennaria* attention was directed to the presence of a true morula as the embryonic stage resulting from cleavage, and also to the entire absence of a stage comparable to a coeloblastula. This is likewise the condition to be found in *Hydractinia* and *Clava*. Rittenhouse ('07) has shown the same to be the case in *Turritopsis nutricula*. It will be shown in a later connection that this is the dominant type of cleavage embryo throughout the entire phylum. At no time is there to be found in either *Hydractinia* or *Clava* a distinctive or permanent cleavage cavity, though there may often be found certain intercellular spaces which are designated as such, but the correctness of which may be seriously challenged. This, again, will be considered in more detail in a later section.

For some time following the apparent completion of cleavage and the establishment of the morula there seems to be a period of quiescence. This is such, however, only in appearance; for in reality there exists a condition of active cell proliferation, as may easily be demonstrated by means of sections of embryos at this time. This has been especially demonstrated and emphasized

in the cases of *Eudendrium* and *Pennaria*, but is no less true in the present instances.

4. *Organization of the embryo.* This has usually been assumed to consist fundamentally in the formation of the tissues, ectoderm and entoderm. In part this assumption is correct, but only in part. For example, the morula may remain for some time entirely devoid of these tissues in any definitive sense, and even in the later larval stage the entoderm may not arise till a late period. As has long been known, among the first evidences of organization is that associated with the formation of the ectoderm. Indeed, this is only what might naturally be expected as one comes to consider the primary function of such a tissue, or its analogue, throughout the animal kingdom. The embryo, no less than the adult organism, requires superficial protection against external conditions. And from protozoon to mammal provision is made to this end by ectosarc and epidermis, and in the embryo by the ectoderm, which may be regarded as the primary tissue of the embryo.

5. *Entoderm formation.* But up to this time there is no definite differentiation of entoderm. It is true, that one will find what has long been designated as entoderm, namely, an interior mass of embryonic matter more or less cellular, but without differentiation of any sort. By some students of hydroid development this condition has been described as the 'end of entoderm formation' (Ende der Entodermbildung). In reality one may better designate it as the beginning of entoderm formation, though even this might be open to question. What we have at this time is simply an interior embryonic mass, often a syncytium, within the enclosing ectoderm, if this be yet differentiated; and of this mass but a very small proportion ever participates directly in entoderm formation. For the sake of clearness it seems desirable formally to recognize this condition by giving to it such name as may express the fact, and at present no better term seems available than 'pro-entoderm.' This only implies the existence at this time of material, a primordium, out of which in varying ways will be developed the definitive entoderm of the larva.

6. *The larva, planula.* The life history of the morula is comparatively brief, perhaps from six to eight or ten hours, the period varying considerably. During this time the definitive ectoderm has been established, cilia developed and the free-swimming larva, the planula, begins its career. Concerning the structure of this organism there is no occasion for special details. It differs little if at all from that characteristic of others whose structure has been repeatedly portrayed, and is too well known to need further account. In the present instance, as in those of numerous others, at the time of the assumption of this condition the larva is still a solid mass, with little organization beyond the above mentioned ectodermal differentiation. A definitive entoderm may not become established till relatively late in larval life, as I have repeatedly pointed out in other cases, and only after a process of physiological differentiation, as shown in a later section. The first evidence of a coelenteron appears as a slit-like cavity in the larval axis, which later enlarges as the reduction and absorption of the pro-entodermic mass proceeds. Finally, by such graduated method does the entoderm become established. At no time is there a mouth or other means of communication with the outside during phases of embryonic or larval history. Planulae of *Hydractinia* have been frequently reared under artificial conditions, and readily transform into the final, or polyp state. Soon after the larva attaches itself the mouth is established by a terminal opening which arises by a rupture and rearrangement of the adjacent cells. Tentacles arise in the usual manner, first three in number, followed shortly by three others at intermediate points, and slightly below the first series. At the base of the polyp there arise root-like stolons, two or more in number, which mark the origin of the hydrorhizal network characteristic of the species.

*C. Clava leptostyla Ag.*

In connection with the work on *Hydractinia* I have taken occasion to re-examine the material upon which was based the work embodied in my previous paper on *Clava* ('06), and have also carefully studied sections of new material which had been fixed in

Bouin's picro-formol, and in Zenker's solution, and carefully stained by several of the most recent methods. So far as it relates to the organization of the egg or its cleavage no occasion has been found for modifying in any essential the earlier conclusions. These I believe to be confirmed in every detail, and lead me to reaffirm the former account. Concerning some few points in relation to the phenomena of maturation and nuclear behavior, including phases of germ-layer formation not touched upon in the previous paper, it is necessary to reconsider and add to the former results.

1. *Maturation.* Concerning the phenomena associated with maturation my observations will be very brief. In the former paper the general facts were explicitly stated and no occasion has been found to call for essential modification. Both in living eggs and in sections of stained material polar bodies were found and described. In connection with earlier accounts of this feature in other eggs of hydroids one may find such expressions as "About this time the nucleus becomes indistinct and finally disappears;" the nucleus "fades from view when the ovum is deposited." These accounts relate almost wholly to observations upon living eggs, and I have repeatedly verified them both in the living, and in sections of fixed eggs. While in themselves such accounts may seem to have little of distinctive value, in a morphologic sense, yet, as expressive of physiological conditions they seem to me to have very large significance. In the first place, these observations described what was actually seen and its fidelity to fact can not be questioned. In the next place, cytological study of fixed material confirms just these accounts. As eggs grow toward maturity the germinal vesicle is large and conspicuous. But as they approach the phase of metabolism involved in maturation a marked change occurs, as is well known. The chromatin network, which has been conspicuous, gradually disappears, and in many cases loses absolutely its affinity for stains. With dissolution of the nuclear membrane, a still further change occurs, which is exactly what these accounts describe, namely, the mingling and fusion of nucleus and cytoplasm to such a degree that it is often difficult to differentiate them by any of the usual methods.

It is just at this time that the maturation phenomena are in process of development. In my previous account some doubt was expressed as to the presence of mitosis. Critical study of fresh material has enabled me definitely to confirm the facts of maturation mitoses attested by Smallwood ('09) in *Hydractinia*, and Beckwith ('09), in *Clava*. A critical review of my earlier material only went to confirm the previous doubt; all of which but tends to resolve the case to one of technique. In the newer material both maturation mitoses were distinguishable without serious difficulty.

2. *Nuclear behavior.* In addition to the foregoing discussion some further reference to points of nuclear behavior seems desirable. In several of my earlier accounts attention was directed to the migration of the nucleus to the distal periphery of the egg as it approached maturity. As is well known, many earlier students of nuclear physiology have sought to correlate directly the nucleus with nutritive functions during the growth period, and its location has been said to conform to this conception, and numerous citations made to facts recorded in phyla above protozoa. So far as the Hydrozoa are concerned I am not able to confirm this view. In the growing oocyte of *Clava* the germinal vesicle is rarely if ever directly contiguous to the nutritive surface of the spadix, and in the period of later growth invariably migrates to the distal surface and comes to lie in immediate contact with the outer wall of the gonophore. While I have not made any attempt at this time to take up the problem for critical inquiry and investigation, yet my general observations tend to render extremely doubtful the view above suggested, at any rate in any very explicit and causal sense. That the nucleus may function in this matter in a general way as in many other vital functions may be probable, yet that its primary or fundamental and direct part in the oocyte has to do with nutritive more than other functions of cell life seems more than doubtful. It may easily be shown that processes of nutrition, along with other phases of metabolism, are functions of the *entire cell working as a whole*. In the earlier paper attention was directed to the phenomena of metabolism as related to the origin and development of the pig-

ment granules of the egg, and it was pointed out that they appeared first in the region of the nucleus, and from this extended as a peripheral zone over the entire egg, the process continuing up to, and even beyond, the phenomena of maturation, which would seem clearly to imply that at most the nucleus was concerned only in the origin of this process, since it early became involved in other functions of very different character.

3. *The chromatin.* In addition to what has been said in this connection as relates to *Pennaria* and *Hydractinia* a few facts may be mentioned as directly bearing on the matter of nuclear fragmentation. In figs. 31 and 32 are shown phases of nuclear behavior associated with maturation. Fig. 31 is a careful drawing of a condition not at all unusual in these eggs. Here one finds undoubted evidences of chromatin fragmentation and dispersal prior to the dissolution of the nuclear membrane. As will be noted, there is as yet no definite disintegration of the nucleolus, which is quite intact, though with a large vacuole. Chromatin granules are variously distributed through the nuclear network, chiefly at nodal points as shown. But the same sort of granules are to be seen just outside the nucleus, and are indistinguishable from those shown in the next figure, in which the nucleus is in process of disintegration, the membrane being entirely dissolved, and the network also surely disappearing. Here also the nucleolus is about to collapse, being flattened on one side, as if ready to go to pieces. Numerous cases of this sort occur in these eggs and seem to confirm what has been said above, that a degree of fragmentation both of nucleus and chromatin is apparently a constant feature. In a few cases I have found these features actively associated with maturation, the first polar body having been already formed.

From this it will be noted that fragmentation of the nucleolus may not occur until that of the nucleus is well under way, as shown in the figures already cited.

I have called attention to the problem of nuclear fragmentation in several of my earlier papers, ('04a, '04b, '06), and in a paper now in press on the development of *Cyanea* ('10), attention is directed to very similar conditions associated with maturation.

It is not a new problem; many investigators having directed attention to the matter. Strangely, however, there has been no very serious attempt to explain its probable significance, further than to suggest, as Wilson had done long ago ('00), its possible relation to the extremely active metabolism involved during the growth period of the egg. Unfortunately, there seem to be serious difficulties involved in such an interpretation; for example, the fact that in many ova growth seems to be almost *nil*. Furthermore, it is not certain that just this type of fragmentation occurs in all eggs just at this period. But whatever may be its significance certain it is that in large numbers of organisms there seems to occur at this time this very interesting fact, that a largely preponderating proportion of chromatin is lost, or at least takes no part in the formation of the chromosomes, and so is a negative factor so far as relates to chromosome function or theory. The bearing of this on the question of chromosome individuality is not without great interest and importance; but no attempt can be made to discuss the problem here. It may be suggested, however, that defenders of the extreme views of chromosome individuality in its morphological sense,—and only in such sense has it any essential significance,—are confronted with a problem, the complexity of which is beyond estimate, and the difficulty of which is hardly less so. Let him who finds difficult the intricacies of 'germ-plasm' hypothesis beware in essaying to unravel the no less intricate mystery, or miracle, of preserving individuality in the metabolic maze through which chromatin must pass in every cytogenic cycle!

In the previous paper (op. cit. '00, , p. 227), attention was directed to the very anomalous aspects of nuclei during early cleavage, features of which were shown in several sketches. Certain of these presented rather strong indications of amitosis, though the condition of the chromatin was such as greatly to obscure the finer details of nuclear structure, and the suggestion was made that "certain phases of the mitotic mechanism may be disguised or actually lacking." As shown above, mitosis has been demonstrated during maturation, yet something of the original doubt remains as to mitosis during early cleavage, the newer material

affording no appreciable advantage over that used in the former instance. At this time the chromatin appears only in the form of extremely irregular, flocculent patches, scattered here and there through the cytoplasmic cell-like aggregates. The same elongated, clavicular, or dumb-bell shaped nuclei previously described are found in the newer material treated by latest methods. Under ordinary treatment the chromatin stains so intensely as seriously to obscure details of structure. Only by prolonged destaining and clearing, and by more delicate staining with picro-hematoxylin has it been possible to reduce somewhat the flocculent aspects. When this is done one may distinguish a granular chromatin constitution, but the chromosomes have defied all attempts to render them distinctive either in form or in number. This relates to conditions in early cleavage, as was pointed out in the previous paper, aspects of mitosis become fairly clear in later cleavage. Beckwith describes mitoses in early cleavage but makes no reference to the anomalous conditions here described.

With all the pains taken in preparation of my material it must be allowed that these conditions are not artifacts, but facts entitled to the same consideration as others of similar treatment. It must be admitted however, that, even at best, our latest refinements as to staining technique must be accepted as only tentatively trustworthy. In other words, it becomes more evident every day that in protoplasmic and nuclear metabolism there are such incessant and intricate variations of chemical conditions that one may not assert that a given stain or fixing agent affords any certain test of a given state at a given time. On the contrary, it will not be denied that a given stain may act in one manner on one cell and on another very differently; or indeed, that it may in another case fail utterly to yield any results whatsoever in differentiation. Under the aspects of chromatin organization, or perhaps better, *lack* of organization, as here portrayed, it has not been possible to obtain any definite information as to the number or character of the chromosomes. But it may be said, as before mentioned, that in *Clava*, as in *Pennaria* and *Hydractinia*, there is an enormous fragmentation and dispersal of chromatin at the time of maturation, most of which must be utterly

lost as chromatin, unless some may be recovered during phases of cleavage, as suggested in the previous paper. Some further reference to this feature will be made in a later section of this paper, in connection with the discussion of theoretical problems involved in the general subject.

Incidentally it may be worthy of mention that in at least one case two germinal vesicles have been demonstrated in a given egg. So far as the writer is aware this is a rather rare occurrence, though probably not more so than that of hermaphrodite gonophores, as described in the previous paper (p. 211). Fig. 23 is a careful camera sketch of these nuclei. There was not the slightest evidence to show that there might have been a fusion of two oocytes in this case, as sometimes happens in other hydroids, the egg being only of average size.

4. *Nucleolar behavior.* In the previous paper (p. 221), attention was directed to some aspects of nucleolar changes associated with maturation. Among these that of vacuolation was particularly mentioned, as was also that of the migration of the nucleolus from the nucleus into the cytoplasm. The latter feature is rather unusual, and is not probably of any large significance. More important is the fragmentation which is a rather common feature. Prior to maturation the nucleolus exhibits various phases of vacuolation. In some cases several vacuoles of slightly differing sizes appear, some of which may later fuse into a larger vacuole. In other cases one finds a single large vacuole which finally occupies almost the whole of the nucleolus, at which time it may happen that the body collapses upon itself, or gradually goes to pieces, i.e., fragments. In other cases there may be in a given nucleus two nucleoli, one highly vacuolated and evidently degenerating, the other having all the appearance of a new organ, without signs of vacuoles.<sup>1</sup> These changes usually occur while the nucleus is

<sup>1</sup> In this case the larger, vacuolated nucleus exhibited a most interesting phase of apparent fragmentation. Almost the whole organ comprised a single large vacuole, and adhering to the outer surface were numerous deeply staining spherical granules borne upon delicate pedicels, the whole resembling a sort of pin-cushion aspect. Just what significance such a condition may have in relation to nucleolar metamorphosis, or its bearing on the problem of chromosome formation, as

yet intact; and in some cases, at any rate, the entire fragmentation, or dissolution of the nucleolus may occur before the nuclear membrane disappears. In other cases nucleolar dissolution and disappearance take place coincident with the nuclear dissolution and maturation, as stated in a preceding section. In some instances the nucleolar degeneration seems to involve a gradual process of shrinkage, probably by solution or absorption by the nucleoplasm. It has been no part of my present purpose to study the matter of nucleolar genesis, or the possible relation of nucleolar metabolism to the genesis and differentiation of chromosomes. An interesting and varied literature on this subject has grown up with recent times, some of which seems to have a profound significance in relation to chromosome theory. But to enter upon this phase would involve time and details far beyond the scope of the present paper.

5. *Later development.* It is not the purpose to enter into any considerable details as to later aspects of development save as they are found to be more or less exceptional as compared with other species concerned. As to cleavage no occasion has been found to modify the account already given in the former paper (pp. 223 to 227). There is much here in common with that known in *Tubularia*, *Hydractinia* and *Pennaria*. While in a certain proportion of the eggs cleavage is more or less regular; on the other hand, in a large proportion, irregularity and lack of order or symmetry is the rule. This is particularly the case with those ova which are flattened laterally against the sides of the spadix of the gonophore. In the case of ova terminally placed in the gonophore the shape is more nearly spherical, and in such cases the tendency is toward regularity. This is what one might naturally expect; yet there are notable exceptions, and one will do well to remember the extremely erratic cleavage of such ova

suggested in a following sentence, I am not prepared to suggest. The nucleus of this egg was in rather typical resting condition, and its chromatin of the usual granular spireme aspect. As stated in another connection, different modes of fixation and staining have appreciably different effects on the egg cytoplasm and nucleoplasm, so that much more elaborate observations would be necessary ere one might venture any very positive opinions on so complex a problem.

as those of *Pennaria*, *Hydractinia* and *Turritopsis*, where the freedom of the egg from all influence of gonophore walls, etc., ought to afford perfect conditions as to regularity of cleavage. It may not be improbable that external conditions of pressure, etc., have an appreciable influence on cleavage, but such facts as those just cited clearly indicate that there are other factors concerned which are probably more potent than the merely physical ones of pressure, gravity, etc.

6. *The morula.* As in *Hydractinia* and *Pennaria*, the early embryo in *Clava* is a morula. Cleavage results in a solid mass of cells, with only incidentally a sign here and there of an intercellular space, and in only rare instances anything comparable with a segmentation cavity. Indeed, one might venture to aver that such cavity is conspicuously absent throughout the ontogeny of this hydroid. As already pointed out, this is not peculiar, but rather the general fact in hydroid development. Something further will be said on this point in a later connection. There is nothing in the morula stage in *Clava* which differs appreciably from that of the other species already referred to. As the embryo reaches the morula condition it assumes the usual spherical shape, whatever may have been the shape of the egg during cleavage or growth. Evidently the walls of the gonophore do not afford any very serious barrier to this change, for one finds all conditions of shape from the flattened lateral pocket of the growing oocyte to the spherical terminal capsule, and the oval capsule of the planula, all derivable in turn from the first as the embryo grows and finally emerges as a pear-shaped planula.

7. *The germ layers.* What has been said on this subject in connection with *Hydractinia* may be affirmed of *Clava*. Granted the assumption of a morula as the primitive embryo, and there is no occasion for question or discussion concerning the segmentation cavity, delamination, multipolar immigration, etc. Absolutely nothing of these is involved in the case under consideration. At the time of the completion of cleavage,—indeed before this, when the morular aspect first begins to take shape,—we have only a spherical cell mass, with syncytial tendencies, and as yet without sign of tissue differentiation. In fig. 29 is shown such

an early morula-like embryo. In this is shown an oval embryo without definitive ectoderm, or sign of entoderm. This condition persists for some time, the only changes distinguishable being that of cell proliferation, or perhaps more precisely, *nuclear* proliferation; for in most cases it is not possible to distinguish the presence of cell boundaries of any definitive sort. But a most remarkable thing becomes apparent under careful staining, —namely, the fact that the internal mass shows a differential staining reaction, represented by the shaded interior. This I take to be indicative of an important physiological change, namely, an incipient entodermal differentiation directly related to the primary purpose of entoderm development, that of digestion. While the results do not as yet warrant a dogmatic pronouncement on this matter, they do tend to confirm a view I have already proposed (cf. *Science*, March 25, 1910). It has generally been assumed that the ectoderm is the primary germ layer, and morphologically this is undoubtedly true. But if the suggestion just made be confirmed by later experiments one will have to aver that, physiologically speaking, the entodermal function is the first to express itself. Further consideration of this point will be deferred to a later section.

a. Ectoderm. The development of this tissue is a graduated process. With the establishment of a surface layer of cells of more or less similar character one is not warranted in designating it as a definitive ectoderm. For, as Rittenhouse has pointed out ('07, p. 453):

Even those cells which are at the surface at the completion of segmentation cannot be regarded as primitive ectoderm, for in the breaking down of the cell boundaries, the formation of the syncytium, and the recasting of the cells, it is quite impossible to say what changes of protoplasm may take place.

Furthermore, it must not be overlooked that, with a primary layer of cells established, there are yet other ectodermal elements to be taken into account, such as interstitial cells, cilia, nettling cells, etc. Only with the formation of the supporting lamella can it be claimed that the definitive ectoderm is really established.

b. Entoderm. As in the case of the ectoderm, what has been said as to entoderm formation in *Hydractinia* will apply for the most part to *Clava*. What has been said above in reference to the morula as the primordial embryo applies in this connection. Entoderm formation is a graduated process, and in its morphology a much slower process than that of the ectoderm. In its physiological genesis it may be said to outrun the ectoderm; for its functions begin almost immediately after the completion of cleavage. As was pointed out in an earlier section, the internal cell-mass included within the primordial ectoderm is not in any sense a tissue, but rather a primordium,—*pro-entoderm*. For some time following the nuclear proliferation of this mass continues. But at the same time another, and extremely different process is under way, namely, that of cellular and nuclear disintegration and destruction. Out of this interior mass relatively few cells will survive to constitute the definitive entoderm of the polyp. What is taking place is in reality a struggle among these cells for nutrition, reminding one of the ingenious theory of Roux ('81), '*Der Kampf der Theile im Organismus*,' though, so far as I am aware, this author never applied his theory in this direction. It is not until after the planula has become free that a definitive entoderm is finally established; indeed, this does not become established until near the metamorphosis of the planula into a polyp, though one may trace stages in the process much earlier. What really happens is that the same sort of vicarious process of nutrition occurs as that by which, in many hydroids, the oocyte grows; that is, the devouring of sister cells or primordial ova; in the later stage occurs the similar process of digesting the pro-entoderm cells and making their substance available as nutrition to the embryo. As is well known, these pro-entoderm cells are richly laden with yolk granules, as were also originally the cells of the ectoderm. But long after the ectoderm has exhausted this primitive supply the entoderm is reducing its surplus cell mass for similar ends.

With the gradual advance of this process the coelenteron of the larva grows larger, appearing in sections as an axial slit of irregular outline, and later assuming a more regular aspect and

becoming more capacious. As the entoderm cells become definitely organized and adjusted in contact with the supporting lamella the entoderm may be said to be established as a tissue. But this does not become complete until a large proportion of the pro-entoderm mass has been reduced and appropriated by the embryo. There yet remains masses of cells in the cavity along with yolk fragments and other debris variously distributed.

Earlier accounts of the differentiation of the entoderm differ in several particulars from that here given. In the first place, it has been generally assumed that the entoderm is early established, an error which I have taken occasion in several accounts to correct. In the next place, the exact mode of its differentiation has not been very critically studied, nor the fate of those parts of the pro-entoderm not directly concerned in entoderm formation. For example, Korschelt and Heider, following the older accounts, have asserted that following the establishment of the entoderm the remaining cell-mass undergoes fatty degeneration, serving in part as food matter, with a residual mass of debris, the fate of which is not formally stated. I have not found in my preparations any evidence of such fatty degeneration, though, as stated above, I have found direct evidence of the operation of digestive ferments. According to Wilson ('83) something akin to amoeboid engulfment of these cells and their intracellular digestion is tentatively suggested:

These appearances suggest, though they do not prove, that the yolk granules and spheroids pass bodily into the cells. I have never seen them in the act of passing into the cells, but the technical difficulties are great, and the other considerations seem sufficient weight to warrant the provisional acceptance of the view advanced.

That something of such amoeboid engulfment may occur is not altogether improbable; though I have found slight evidence of it. We know that in the growth of the oocyte in certain species just such a process does take place, and its occurrence in the slightly later history of the embryo would be what might naturally be expected. Indeed, I have, in an earlier part of this paper, suggested such a process in the behavior of the cells of the pro-

entoderm during differentiation. But associated with the process there are strong evidences of the action of digestive ferments which are set free by these cells in which this process is first set up and carried forward. This likewise takes place in the case of the oocyte during growth, as has been shown by many recent observers. The suggestion of Metschnikoff long ago, that intracellular digestion forms the dominant, if not the only digestive process in coelenterates, is not borne out by recent investigations. For example, it is well known that medusae, actinians, etc., capture highly organized prey, such as crustacea, fish, etc., and digest it quite after the fashion of the higher Metazoa. The same thing is easily demonstrated in hydroids, in which small organisms, like worms, copepods, etc., form an important food supply. Gland cells are well known in the entoderm of Hydrozoa and are evidently associated with digestive functions. Hence it seems more than probable that enzyme digestion is no less a feature in this than in other animal groups; and that it more than any other, is the mode involved in the reduction of this inner cell mass of the planula is almost certain. This in no wise contravenes the fact of the presence of yolk granules in the entoderm cells, for they were original constituents of these cells, just as in the case of the primordial ectoderm cells. Whether such yolk granules are ever taken in entire by the larval entoderm may be open to doubt, at least till better sustained by direct evidence than at present.

So far as I am aware, the general conception herein outlined as to the physiological processes involved in this phase of larval development has not been hitherto proposed. Of its fundamental validity there seems no serious objection and much direct evidence. In brief, it involves these facts: First, that of the pro-entoderm mass of cells relatively few go to constitute the definitive entoderm of the planula. In the second place, the primary process involved in the necessary differentiation must be one of selection. So far as one can distinguish these pro-entoderm cells are alike in form and potency. The primary demand in embryogeny is growth, which involves nutritive material in some form. And the only source of such is that associated with these cells

as yolk granules, which can only become available by the dissolution of the cells which contain them. Supposing that originally it was equally distributed, it could only remain so by the further assumption that cell division was likewise equal and continuous throughout. This we know is seldom the case, being in general inversely as the amount of yolk varies. Hence those cells whose growth and metabolism became more rapid would first exhaust their own deutoplasm and demand supplies from outside. And here must originate the struggle among cells which has been emphasized above.

Assuming the substantial truth of the conception we must face the implication that the older views as to the ontogenic and phylogenic significance of the germ layers are discredited by these further facts, as they have also been in theory. I believe we may, therefore, conclude that fundamentally the phenomena involved in germ-layer formation are primarily physiological processes, and relate to protective, motor, and nutritive ends; and that only secondarily, if at all, can they be supposed to have any significance in ontogeny or phylogeny.

#### REVIEW AND DISCUSSION

As stated in an early section of this paper one of the purposes in view was to review certain phases of current and earlier theory and doctrine concerning problems of ontogeny, in the light of recent knowledge, and to seek to point out and correct such errors as may easily come within the scope of pertinent discussion. This seems to the writer particularly important and desirable just at this time of virile criticism and readjustment.

For some time the conviction has grown that not a few of the earlier views and theories touching ontogenetic problems had outlived their days of service, and that new facts were demanding new methods of interpretation. For example, who today pretends to invoke, in its original content, the Recapitulation Theory in correlating ontogeny and phylogeny? Who would seriously defend, or use the so-called laws of cleavage in interpreting every phase of egg development? And so one might multiply examples.

The fact remains, however, that just these outgrown systems or theories still cumber the literature, the available text-books and manuals for introducing students to these subjects of present day biology, much to the reproach of its leaders and sponsors.

With the desire to aid, in however small a degree, in correcting phases of error, or what appear such, the writer will aim under this section of the paper briefly to pass in review the chief aspects of the problems involved, and, so far as may be practicable, will endeavor to show distinctive examples of inadequate theory and erroneous implications and deductions.

### *1. Origin, multiplication, and growth of germ-cells*

It seems worth while briefly to summarize results which observations, more particularly my own, have brought to light on these several aspects of ontogeny. Many of the facts have already been made known in previous papers, but care will be taken to avoid, as far as may be, any unnecessary duplication, giving attention chiefly to those features relating to phases which seem to call for consideration. Concerning the earlier controversy as to the mere place or tissue in which the germ-cells arise, it is no longer necessary to multiply words. Recent work from various sources, and especially that of Goette ('07), has, I believe, placed the subject beyond further dispute. That there is any such region as may be designated a 'Keimzone' or 'Keimstätte' may be at once dismissed as absolutely without warrant as a general proposition. Furthermore, that the germ cells have their origin in the ectoderm alone in hydromedusae may be similarly denied and dismissed as unworthy of further inquiry or doubt. And still further, I am thoroughly convinced that the still more recent controversy as to the hypothesis of the 'germ-plasm,' if not as clearly a delusion as the preceding, is yet without the slightest support from the ontogeny of the group under review.

It is a matter of easy demonstration that in many species of hydroids the egg may be followed in every detail from its origin as an ectoderm or an entoderm or interstitial cell through its gradual differentiation and growth to maturation, as a distinct individual

cell, without the slightest tendency to multiplication. That is to say, in species of Eudendrium, Hydraetinia, Campanularia, Pachycoerulea, and others, there is at no time any organ which is ovarian in character, within which masses of primordial ova arise and pass through oogonial and oocytic phases familiar in other species to be mentioned later; but a given cell of the entoderm which is to give rise to an egg begins to grow, and either *in situ* or after migration into the gonophore, develops directly into a typical egg, and later, after fertilization, gives rise directly to an embryo and finally to an individual polyp. On the other hand, in many cases, e.g., Pennaria, Tubularia, Syncoryne, Hydra, large numbers of primordial ova arise in what may be regarded as an ovary where, by a series of cytological changes, they exhibit the oogonial and oocytic phases referred to above. These somewhat strikingly different modes of oogenesis may, for convenience be designated as the 'direct' or 'individualized' and the 'indirect' or 'oogonial' modes. That they are sharply distinct, or qualitatively differentiated types of oogenesis is not claimed. In this, as in other phases of development, there are all shades of intergradation and relation to be found in these and other species of Cnidaria.

Correlated with these apparently widely divergent modes of origin are those of nutrition and growth. In the 'direct' or 'individual' ova nutrition is almost invariably likewise through the direct medium of the adjacent tissue cells, which supply by diffusion the appropriate nutritive plasma. On the other hand, in ovarian eggs, which involve oogonial and oocytic generations, there arise indefinite masses of primordial ova; and the growth of certain of these as ovarian eggs, is largely through the active appropriation of the excess primordial ova, which are literally devoured whole, or predigested to a liquid plasma, which is then absorbed. Illustrations of both these processes are too familiar to call for special emphasis. While the two processes of nutrition are thus apparently different, intermediate cases are not unknown, e.g., Eudendrium hargitti, recently described by Congdon ('06, p. 39) has been found to comprise something of both modes. And, though it belongs to a genus in which oogenesis

is associated in its nutritive relations with the direct activities of the tissue cells of the parent organism, yet in this particular species the egg certainly turns parasite, if not cannibal, and devours bodily the cells of either ectoderm or entoderm as may happen to afford it particular support at a given time. And one finds in these growing ova of *E. hargitti* eggs literally loaded during most active growth with the engorged nuclei of tissue cells, the exact counterpart of those conditions found in *Pennaria* and *Tubularia* in which the growing eggs are similarly packed with the primordial ova of the ovarian tissues.

In his earlier studies on heredity Weismann admits that germ-cells may be derived from somatic cells, *e.g.*, (*Essays on Heredity* 1, p. 209):

It is quite impossible to maintain that the germ-cells of Hydroids or of the higher plants exist from the time of embryonic development, as indifferent cells, which cannot be distinguished from others, and which are only differentiated at a later period. Such a view is contradicted by the simplest mathematical consideration; for it is obvious that none of the relatively few cells of the embryo can be excluded from the enormous increase by division, which must take place in order to produce the large number of daughter individuals which form a colony of polyps. It is, therefore, clear that all the cells of the embryo must for a long time act as somatic cells, and none of them can be reserved as germ-cells and nothing else; this conclusion is moreover confirmed by direct observation.

In later discussing this feature, while still contending that in most cases germ-cells arise early in ontogeny, Weismann is yet compelled to admit that in Hydrozoa these do not arise till very late, and indeed in individuals of a later generation, (*Evolution Theory*, vol. 1, p. 410). Notwithstanding this admission he still contends for his dogma of germinal continuity:

Here the primordial germ cell is separated from the ovum by a long series of cell-generations, and the sole possibility of explaining the presence of germ-plasm in this primordial cell is to be found in the assumption that in the divisions of the ovum the whole of the germ plasm originally contained in it was not broken up into determinant groups, but that a part, perhaps the greater part, was handed on in a latent state from cell to cell, till sooner or later it reached a cell which it stamped as a primordial germ-cell. Theoretically it makes no difference whether these germ-tracks, that is, the cell generations which lead from the ovum to

the primordial germ-cells, are short or very long, whether they consist of three or six or sixteen cells, or of hundreds and thousands of cells. That all the cells of the germ-tracks do not take on the character of germ-cells must, in accordance with our conception of the maturing of determinants, be referred to the internal conditions of the cells and of the germ-plasm, perhaps in part also to an associated quantum of somatic idioplasm which is only overpowered in the course of the cell divisions. This splitting up of the substance of the ovum into a somatic half, which directs the development of the individual, and a propagative half, which reaches the germ-cells and there remains inactive, and later gives rise to the succeeding generation, constitutes *the theory of the continuity of the germ-plasm* (p. 411).

Theoretically, the hypothesis is interesting and developed with much plausible argument. Yet its demonstration is far from evident, indeed quite beyond demonstration, as has been frequently pointed out by many of his critics. However, Weismann insists that there are evidential facts:

The hypothesis does not depend for support merely on a recognition of its theoretical necessity, on the contrary there is a whole series of facts which may be adduced as strongly in its favor. Thus, even the familiar fact that excision of the reproductive organs in all animals produces sterility proves that no other cells of the body are able to give rise to germ-cells; germ-plasm cannot be produced *de novo*.

It is passing strange that he should ignore the body of facts concerned in regeneration, and among them the reproductive organs. And it is still more strange that in support of this he should cite in detail the Hydrozoa as illustrating and supporting the hypothesis, ignoring the well-known facts that among these are abounding evidences which afford insuperable objections to just these assumptions. The present author has, in many cases, shown that gonads may be as readily regenerated by hydroids and medusae as any other organs; and that not for once or twice, but repeatedly in the same specimen, and that *de novo* and *in situ*; not the slightest evidence being distinguishable that any migration through pre-existing 'germ-tracks' occurred. The assumption that in these animals the gonads have "been shifted backwards in the course of phylogenetic evolution, that is, have been moved nearer to the starting point of development" seems so at variance with known facts as to be difficult to appreciate

or respect. That "the adherence of the sexual gonophore to the hydroid colony has made a more rapid ripening of the germ-cells possible," or that "nature has taken advantage of this possibility in all cases," as claimed by Weismann, is but another example of subservience to theory; for I cannot believe he can be ignorant of the general fact that there is not the slightest evidence that in hydroids with fixed gonophores the germ-cells ripen more rapidly or more frequently.

It is in vain to attempt to bolster up these speculations by cleverly designed diagrams; for such devices are too often mere products of a vivid imagination. Furthermore, it is difficult to account for the dogmatic *persistence* with which this writer seeks to sustain the view that the germ-cells originate exclusively in the ectoderm. In the earlier work, which makes up his splendid monograph already referred to, he has admitted again and again the probable origin of the cells in the entoderm (pp. 215-217). But in his 'Evolution theory' (p. 415), it is asserted, "in no single case is the birthplace of the germ-cells to be found in the entoderm, but always in the ectoderm, no matter how far back it may have been shunted." And in citing cases in support of the point he refers to *Hydractinia* and *Podocoryne*, both of which are known to prove the exact opposite, as shown by Bunting ('94) and Smallwood ('09), as well as by the writer in numerous similar cases.

The following critique by Lloyd Morgan ('91) is pertinent in this connection:

This germ-plasm resides in the nucleus of the cell; and it would seem that by a little skillful manipulation it can be made to account for anything that has ever been observed or is likely to be observed. It is one of those convenient invisibles that will do anything you desire. The regrowth of a limb shows that the cells contained some of the original germ-plasm. A little sampled fragment of *Hydra* has it in abundance. It lurks in the body-wall of the building polyp. It is ever ready at call . . . . . Now, although I value highly Professor Weismann's luminous researches, and read with interest his ingenious speculations, I cannot but regard his doctrine of the germ-plasm as a distinctly retrograde step. His germ-plasm is an unknowable, invisible, hypothetical entity. Material though it be, it is of no more practical value than a mythical germinal principle. By a little skillful manipulation,

it may be made to account for anything and everything. The fundamental assumption that whereas germ-plasm can give rise to body-plasm to any extent, body-plasm can under no circumstances give rise to germ-plasm, introduces an unnecessary mystery . . . . The fiction of two protoplasms, distinct and yet commingled, is in my opinion, little calculated to advance our knowledge of organic processes.

It has been assumed, as the foregoing citations clearly show, that there is some predetermined order of sequence and relation as to the origin, nutrition, growth, etc., of germ-cells, not only in such a group as the hydrozoa, but throughout the animal kingdom. And with this as a postulate assiduous search has been directed to its support. It is not necessary that one should, *a priori* discredit the *method*, for it is perfectly scientific,—within limits. The fault which must be emphasized is that it has been so conspicuously *partial* and *dogmatic*. Facts quite as accessible, quite as convincing, have been silently ignored; and it is thus that such work or method becomes both *unscientific* and *untrue*. I believe the foregoing facts must suffice to show that, both as to origin, differentiation and growth, the germ-cells of the Hydrozoa, so far from sustaining the doctrine of the germ-plasm, afford the strongest and most direct evidence to the contrary.

## 2. *Doctrines of homology*

If one were asked to indicate the dominant conception which characterized the biological activity of the greater part of the nineteenth century he could hardly go far amiss in phrasing it somewhat as follows: *The perennial and irrepressible search for homologies!* This would be confessedly the case with so much of the period as comprised the Darwinian epoch of biology. But the conception belongs quite as properly to the seething period of the biological renaissance of the early half of the century, and finds expression in the researches of von Baer and Cuvier, Lamarck and St. Hilaire, and a long roll of hardly less distinguished names. But strangely enough the doctrine had antipodal significance under the early, as contrasted with the later epochs of thought. To the first homology embodied the postulate of *types of creation* according to the conception of 'archetypes' of plan and

structure, details of which have been elaborately developed by Owen ('48) whose well-known 'Homologies of the vertebrate skeleton' is its best expression. But to these naturalists homology meant *likeness of structure merely*, with the implications of ideals and design. To naturalists of the later period the conception took on an infinitely larger scope and significance. Like the former, they were free to accept likeness of structure as an index of homology; but following the blazed trail of Lamarck and St. Hilaire, they conceived in the doctrine the *key to lineage*. To them homology involved kinship; and 'archetypes' as such had no vital meaning. It is not strange that, under the masterful hand of Darwin, the newer doctrine gave to biologists a working hypothesis comparable with that of gravitation, and at once placed biology on the foundation of scientific certitude.

To naturalists of both periods must be ascribed well deserved praise. Both sought in the most conscientious and critical manner to discern the facts of homology. Among both were those of divergent and conflicting views, von Baer and Cuvier versus Lamarck and Hilaire; Agassiz versus Darwin. In both were elements of important truth; in both were extremes of mischievous error. It is not the purpose to undertake any critical review of the phases of conflict involved in these antithetic aspects of one of the most profound of biological doctrines; but rather, ignoring extremes of the earlier period, whose errors have largely gone into oblivion, to point out in briefest way wherein, under the ardent impulse of the newer view, something of extravagant over-valuation has come to have a retarding and mischievous influence upon biological thought and progress. It hardly need be said that in this matter attention will be directed to those points in particular which have come under my own lines of research. A similar duty has been ably performed upon a larger scale by several brilliant authorities, among them Wilson ('94), Morgan ('03), Montgomery ('06).

a. *The germ-layers.* No occasion exists for a review of the origin of the conception of germ layers developed through the work of Wolff, Pander, von Baer, Remak and Kölliker. It is sufficient to my purpose to cite the astute observation of Huxley

as to the likeness of the diblastic tissues of coelenterates and the mucous and serous layers of the embryo ('49). Let it be noted, however, that Huxley does not designate these as *homologous*, but rather as *analogous*. Ten years after his first utterance he remarks "It by no means justifies the assumption that the Hydrozoa are in any sense arrested developments of higher organisms. All that can be justly affirmed is, that the Hydrozoon travels for a certain distance along the same great highway of development as the higher animals." (Oceanic Hydrozoa, p. 2.)

Interestingly enough, the embryological researches of the time, led by Kowalevsky, Gegenbaur, Haeckel and others, centered about this pregnant conception of Huxley and led Haeckel to formulate his famous Gastraea Theory, with all its far-reaching implications as to the homology of the germ layers of all embryos, "from the lowest sponge to man." Of course, the gastrula at once sprang into a position of commanding importance in embryology, and as the prototype of Haeckel's hypothetical gastraea became a focal factor in embryological thought for a whole generation. It is not strange, therefore, that the Coelenterata, as the distinctively diploblastic phylum of the animal world, should early come in for a more than usual measure of interest and concern; and as the theoretical ancestral phylum from which all higher metazoa must have arisen, should have at once assumed a unique and dominant phylogenetic importance. When, however, it is clearly known that in only a single class of coelenterates does gastrulation occur, and that in no case is the gastrula, as an embryo, known, it seems remarkable to the point of surprise that the theoretical postulate should still be cherished by not a few students of phylogeny. Current literature, however, furnishes abundant evidence of just such adherence to tradition.

b. *The planula.* As is well known the planula is the distinctive larva of the entire phylum, including also the sponges. It has generally been assumed that the planula is a specialized gastrula, and that in some early species its enteron must have been formed by gastrulation. In this again there is involved the further inference and implication of the dominance of the biogenetic

law. Granted the diploblastic character of coelenterate and sponge; granted further, the gastrula stage in ontogeny throughout a large proportion of higher Metazoa, who could well resist the conclusion jumped at by Haeckel as to the necessary homology of gastrula and planula, facts to the contrary notwithstanding!

*c. The morula.* It has just been stated that the planula is the distinctive larva of coelenterates. Another ontogenetic stage, however, must not be overlooked, namely, the morula. Of this one hears little now-a-days, though formerly it was a name fairly common in the literature of embryology. Even so recent a text-book as that of Korschelt and Heider devotes to it a single brief paragraph or so. They remark, "we shall see that examples of such a mode of origin of the two primary germ-layers are still ascribed to many Hydroids and Anthozoa, though probably the greater part of the cases referred to this method can be reduced to epibolic gastrulation, in which events the morula stage, as being a schema founded on erroneous assumptions, would have to be omitted." As an illustration of subserviency to dominant theory this sentence is a brilliant example! As a matter of fact epibolic gastrulation is absolutely unknown in coelenterate development, cases given of its occurrence having, without exception been proven egregious errors.

It might be questioned whether the morula, as a stage, should be given recognition. But when it is taken as the counterpart of the blastula, a stage everywhere recognized, but comparatively rare in the phylum under review, the objection vanished. The morula is far and away the dominant cleavage embryo in Hydrozoa and common in other classes, the Scyphozoa alone excepted. Accounts of its structure and origin given in an earlier section obviate any call for details here. Suffice it to say, that the complicated methods described by Metschnikoff ('86, p. 70), while interesting and ingenious, are but of small value. That delamination and immigration (polar or multipolar), may occur need not be questioned; but that they occur in any such degree of frequency or constancy as to constitute laws of entoderm formation none who has had to do with the problem would hesitate to deny.

While less importance is attached to this problem of germ layers

than formerly, one still finds it more or less dominant in embryology. In his book, 'The Development of the Frog,' Morgan ('97) gives the subject usual attention; and in the still more recent book, 'The Development of the Chick,' Lillie ('08) devotes several pages to the subject, and it crops out repeatedly in the earlier chapters. The germ layer theory came to have a larger place than might otherwise have been the case in the attempt to discover some ultimate embryological basis for homology, and similar warrant for the so-called Biogenetic Law, or Doctrine of Recapitulation.

It has long passed as a cardinal doctrine in embryology that the primary germ layers form a constant, and more or less infallible basis for homology,—a sort of court of last appeal where other criteria fail. But not a few recent results have tended to force the concession that even here there have been hasty generalizations. Not only in modes of formation and development have the germ-layers been found to differ widely, but in their function and fate in ontogeny there has likewise been obvious variation and discrepancy at many points. Balfour long ago called attention to discrepant modes of mesoderm formation, and recent experimental results have shown that organs of usual ectodermic origin are far from dependent on such mode of derivation. In coelenterate ontogeny the most radical divergences as to modes of origin are too well known to call for extended review. From the extreme mode of delamination exclusively in entoderm formation as pointed out by Metschnikoff in *Geryonia*, and since confirmed in substance by Brooks ('86), who calls it "very peculiar, and without any exact parallel," to that of gastrulation in *Seyphozoa*, with its confusing variations and exceptions, which involved those rancorous discussions of Claus, Goette, and others, and the more usual mode through the morula, the entire gamut of germ-layer formation might seem to be epitomized. But despite the misguided and essentially mischievous (however well meant), efforts to derive all these phases from a mythical *gastraea*, now long a discredited and discarded phylogenetic monstrosity, the fact remains that there is probably no genetic relation whatsoever between them.

d. *The blastocoel.* As another phase of the germ-layer problem, the *cleavage-cavity* calls for some passing notice. Formerly it had large attention at the hands of embryologists, and, though less emphasized at present, it has not passed out of consideration. One can hardly consult a current paper dealing with early development without meeting the problem of the origin of the cleavage cavity and its later fate in ontogeny. It is not necessary that one should assume to discredit entirely any possible morphogenic significance to this cavity in any group of organisms; but one does not need to study any considerable series of ontogenies to have forced upon him the conviction that its importance has been greatly exaggerated and correspondingly misinterpreted. One of the first impressions to be gathered from any considerable comparison of coelenterate embryology is that of the conspicuous absence of any definite blastocoel. It is only necessary to cite such figures as those given on plates I to III, illustrating these phases in *Pennaria*, *Tubularia*, *Clava*, *Hydractinia* to make this point very evident. It is true that here and there at certain stages of cleavage may be found irregular intercellular spaces which have been designated in general as segmentation cavities by those describing them. *Spaces* they undoubtedly are; but they are not cavities which have any permanence, either of form or position, but shift, or disappear under the erratic adjustments of the blastomeres; and one might about as well speak of the morphologic significance of the interstices in a box of oranges or bag of potatoes as of these promiscuous intercellular spaces.

Another feature may also be mentioned in this connection. That is the rather significant fact that in many species, such as *Hydractinia*, where during very early cleavage a cavity may appear incidentally, it almost immediately disappears, becoming totally and permanently obliterated by encroaching cells. And even in certain species, where a more or less characteristic cavity arises and persists for a time, as in certain *Geryonids*, I am constrained to interpret it as having a physiological rather than morphological significance,—a sort of embryonic receptacle for the deposition of cytolymph, or other substances developed during cleavage, or *possibly* for food matters derived from the water

during early cleavage, or even later the retention of infusoria, as claimed by Merejkowsky ('83), though this may be doubtful.<sup>2</sup>

Hence the facts herein adduced, together with the further fact of its extreme variation as to size, shape, position, or, still more significantly, its absolute absence in a large proportion of the species of the entire phylum, afford ample warrant for the conclusion that, so far from having any necessary morphogenic or ontogenic significance, the blastocoel may be said to be absolutely devoid of anything of the sort, least of all of any relation to phylogeny.

*e. Cleavage homology.* With the later development of the doctrine of homology there came to be involved varying phases of embryology, as shown above. One of its latest aspects is that concerned with cleavage, which has assumed a place of commanding influence within recent years, as expressed in the flood of literature which sprang into existence dealing with the subject from every point of view,—normal, artificial, experimental. Conklin ('97), has stated well the subject as follows:

In the whole history of the germ-layer theories I see an attempt to trace homologies back to their earliest beginnings. This problem is as important today as it ever was, and whether one find these earliest homologies in layers or regions of blastomeres or the unsegmented ovum itself, the quest is essentially the same. Within this question of the earliest homologies is included another of great and present interest, viz., the significance of cleavage.

With the broader implications and relations of this subject there is neither the time nor occasion for extended review in

<sup>2</sup> I can but express the strong conviction that those who contend for the presence in such cases of a definitive segmentation cavity and blastula are in serious error. It seems not at all adequate to aver that the absence of any true blastocoel is due to the 'abbreviation of this stage of development,' as G. T. Hargitt ('09) has designated it. As suggested above, but for the earlier theoretical significance involved in the matter, it may be doubtful whether any such contention would be made as that under review. To the writer it seems a pity to waste words over the subject in the form of argumentation. The facts are their own best exponent, and with these clearly apprehended there ought to be small occasion for controversy. The presence or absence of syncytial conditions has nothing whatever to do with the problem. Long before a syncytium has developed the morula has arisen as shown above, a fact as incompatible with the blastula as the planula is independent of the gastrula.

this connection. With certain limited aspects of the problem as they relate to coelenterate ontogeny facts have come to knowledge which demand consideration. In a general way it may be said of the problem of cleavage homology that two rather divergent schools of biologic thought have grown up. One of these, ably represented by Driesch and O. Hertwig, maintain that cleavage is a more or less general and quantitative process, the resulting blastomeres being largely equipotent in later development, their individual values depending largely upon relations of position, etc. The other wing of thought would hold that cleavage is fundamentally a qualitative process, involving a nicely predetermined and 'orderly sifting of materials,' resulting in a splendid 'mosaic work,' each cell fitted into its predetermined place with mathematical precision. Under the latter conception 'cell lineage' became the dominant problem of embryological research.

As a corollary to this, not only were blastomeres factors of supreme concern, but the natural and almost necessary implication followed that there must of necessity be predetermining factors in the unsegmented egg even more fundamental than those in the blastomeres. Hence came into prominence the search for evidences of 'formative stuffs', 'prelocalized germinal areas', etc. Waiving all further consideration of this particular aspect of the problem in its theoretical implications, I may very briefly cite facts concerned with coelenterate cytology, and attempt to show their bearing in the case.

In earlier contributions on the subject of cleavage, particularly in *Pennaria* and *Clava*, and further facts given in previous parts of the present paper, attention has been directed to facts which must be their own exponents. As to any blastomere homology in any of these cases it is difficult to conceive. Furthermore, both under normal conditions and through experiment, it has been demonstrated over and over that one or many blastomeres may be detached without in the least modifying the course of development in any particular. With such pictures as those in figures 1 to 30 before one, he would need be possessed of a measure of imagination beyond compare who could discern any sign of a

'mosaic work'! And what shall be said as to the existence of prelocalized germinal areas in such ova? I have searched throughout the phylum for ova having any semblance of such, but without evidence of its existence. It was thought for a time that *Clava* might be a case, but the most painstaking efforts to detect it were only negative. For a time Conklin believed he had found such in the ova of *Linerges*, and so pronounced; but his final utterance ('08, p. 166), recalls this: "The view expressed in my preliminary note on the development of *Linerges*, that the three layers of the egg give rise to the ectoderm, the entoderm, and the mesogloea is not confirmed by further study."

That there may be certain special distribution of egg *material* I have already shown in the case of several hydromedusae, but this is by no means implies that it is *germinal* in character or definitely prelocalized.

### 3. *Amitosis*

In several earlier papers I found occasion to call attention to what seemed to be amitosis in cells during early cleavage. In several of these the evidence seemed direct and positive; in others the indications were somewhat general and indirect. The fact that several later students of cleavage in eggs of hydroids failed to confirm my results, while in the case of several others there has been very explicit confirmation, leads me to briefly review the matter as it appears at the present time. As I have elsewhere stated, the *question of amitosis is purely one of fact*. Whatever may be the implications of amitosis in its theoretical bearings on problems of heredity or otherwise, it must be evident that to attempt to discredit it on such grounds, or others of like nature, can only result in confusion worse confounded. One fact is just as sacred as another, and just as much entitled to respect and consideration, and is bound sooner or later to be taken account of. The extreme attitude of Ziegler, Vom Rath, and certain other cytologists who would have us believe that amitosis is to be found only in senile or pathologic tissues, will have to be abandoned as altogether unwarranted. Cytologists no less capable and conscientious, in growing numbers, accept amitosis as a normal

and not rare mode of cell division. The following recent utterance of one of the avowed conservatives will show how just is this claim: "Accepting the idioplasm hypothesis . . . what do we know of its transmission? We may answer with assurance that it is transmitted from cell to cell by division; and we may safely presume, I think, in most cases by mitosis, though the direct or amitotic process may play a larger rôle than was formerly supposed." (Wilson, '09.)

My first suggestion concerning the problem was made in connection with my early account of *Pennaria* ('00); and the same year, Allen, one of my graduate students, made a similar statement in connection with the development of *Tubularia*. In a paper on regeneration, my son, G. T. Hargitt ('03), described abundant amitoses in the regenerating hydranths of *Tubularia*, and suggested the probable relations of the process to rapid growth and metabolism. In several contributions Child has also described amitosis, and in one in particular ('07), gave a brief account of the process in a series of organisms from coelenterates to birds. In one of these he made bold to predict that "future investigations will probably show that amitosis is at least as important in the life of the cell as mitosis." How timely was this prediction may be inferred by an examination of several recent papers on the subject, particularly by Patterson ('08), on 'Amitosis in the pigeon's egg,' and Glaser ('08), 'A statistical study of mitosis and amitosis in the entoderm of *Fasciolaria*.' In both these studies it is interesting to find so striking a vindication of Child's forecast. Patterson finds that at certain stages amitosis is quite as common as mitosis; and suggests "it seems very probable that amitosis is the result of special physiological conditions, which create a stimulus to cell division, . . . whatever factors are involved in bringing about the rapid growth of any region would seem to be concerned in causing amitosis." This affords an interesting agreement with the suggestion made by G. T. Hargitt, as quoted above. Glaser also concludes "that amitosis plays in this instance" (*Fasciolaria*) "an important, if not the chief part in the differentiation of a definitive tissue."

These several series of facts afford, as I believe, very strong confirmation of my own results as related to Eudendrium, Pennaria and Clava. They are also further supported by interesting results described by Young ('08) in connection with the 'Histogenesis of Cysticercus pisiformis.' In this paper is found the somewhat radical suggestion that cells may arise *de novo*, from a 'cytoblastema,' much as held by Schwann long ago.

It is not necessary to review this phase further than to point out its relation to a similar suggestion made by the writer concerning a somewhat similar origin of cells after nuclear fragmentation in both Eudendrium and Clava. Several of Young's figures are strikingly similar to those given in connection with Eudendrium.

Another most interesting confirmation of my results is to be found in the account of Brooks and Rittenhouse ('07), of the development of Turritopsis. In this case both mitosis and amitosis are found occurring 'simultaneously in the different cells of the segmenting egg.' The varying size of the amitotic nuclei and their reticular structure, confirm with utmost exactness my earlier accounts. Furthermore, the association of amitosis with an approach toward syncytial conditions also resembles the condition in Eudendrium, as does also the metabolism associated with yolk digestion. I cannot agree, however, with the authors that there is any such relations involved in any of these processes as would bring them into conformity with the theory of Flemming, Ziegler, and others, that they presage degenerative ends. On the contrary, they seem to me to be most intimately associated with the intense metabolism and rapid growth of histogenesis.

It remains briefly to refer to phases of nuclear behavior so characteristic in the cleavage of Pennaria, and to a less extent in Clava. Among these are such features as the highly vesiculate aspects of the nuclei during early cleavage, and the equally anomalous features of clavicular, reniform and dumb-bell shaped nuclei. These facts have been very abundantly confirmed by the several researches of Hargitt, Smallwood, and Beckwith, already cited. Their interpretations, however, differ very widely from my own, and with plausibility and force. At the same time I fail to per-

ceive that the facts are not quite as clearly within the amitotic mode, and with apparently quite as strong evidence in support of the latter view. Granted the facts of amitosis as a normal process in cytogeny, and this is no longer open to denial, its occurrence along side of mitosis must be allowed. And even where one investigator may find mitosis, another may find both mitosis and amitosis; and this I have shown in the cases already cited, and my results have been confirmed in similar cases by others.

But there is still a further word in this connection. The fact that the nuclear vesicles differ so markedly in size, shape and number is rather difficult to interpret on the basis of mitosis alone. Are these several vesicles derived from single chromosomes, or from several which have fused? If the latter, how shall we correlate the fact with the further fact, admitted by both Smallwood and Beckwith, that it is not essential that these vesicles should fuse between successive mitoses? But how then shall we attempt to explain the assumed exact nuclear equivalents of every mitotic division? But if, on the other hand, it be held that these nuclear vesicles are originally derived from single chromosomes, as seems more likely, how are we to account for the marked diversity of size, number and shape? These queries are not suggested out of any captious spirit, nor on the other hand, as affording an insuperable objection to the interpretations given by these authors, but as more or less clearly pertinent questions which warrant consideration in connection with the problem concerned.

The further assertion of Smallwood in this connection that "the mere shape of the nucleus in *Pennaria* is no indication of amitosis," may be looked on as somewhat of an evasion of the real issue. I have nowhere made such a claim; but if such were the case it might with pertinence be replied that *mere shape* is not the point at issue. On the other hand, we are here concerned with *particular* and *anomalous* shape, a very different matter. Whether shape has any significance in this relation depends to a marked degree upon the *kind of shape*. That a reniform, or dumb-bell shaped nucleus 'is no indication of amitosis' may be flatly denied, where it is more or less prevalent. *Given such shapes*, while

spheres, spindles, etc., are held to be types of normal nuclei, I think it must be allowed that the burden of proof that the former are but phases of the latter is upon the champions of exclusive mitosis, and thus far the evidence which they submit has not been convincing.

With the facts herein presented, and the cumulative evidence available from a wide range of observation and authority clearly appreciated, it seems difficult to evade the force of the conclusion which is implied. The writer believes, therefore, that his earlier tentative suggestions concerning amitosis as a mode of nuclear activity is not only not discredited nor disproved by later researches, but is rendered both credible and probable.

#### SUMMARY

The main points embodied in the paper may be summarised as follows:

1. Later observations on the development of *Pennaria*, and including a new species, go clearly to confirm the earlier results, and to show that it is not peculiar to a single species or to a given locality.

2. Observations on the development of *Hydractinia echinata* also confirm much of that found to occur in *Pennaria*, including cleavage, ectosarcal features, formation of germ layers, etc.

3. The same may be said in general as to *Clava leptostyla*. New facts as to certain histogenic aspects seem established, and the significance of the early embryo,—the morula,—is emphasized.

4. Concerning the origin and growth of germ cells it becomes more and more certain that the theoretical contentions of Weismann find no warrant in the ontogeny of Coelenterates, and particularly in that of Hydrozoa, the group especially claimed by him.

5. A review of earlier doctrines of homology goes to show that they have been greatly overestimated as criteria of phylogeny. This includes especially the features involved in germ layers, the early hydroid larva, cleavage homology, prelocalization of ger-

minal areas, etc. The facts of homology and ontogeny as related to phylogeny leave much to be desired ere it will be possible to sustain the earlier conceptions of recapitulation and its enormous implications as to biological philosophy.

6. Amitosis as a factor in cytogenesis is a question of *fact*. Cumulative evidence from almost every field of cytology goes to show that it is neither rare, nor limited to senile or pathologic conditions of cells or tissues. Its significance in cytogeny is difficult to overestimate. It is not unknown as a factor in embryogeny in any of the great phyla of nature. *As a fact* it is no less sacred than any other, and must be reckoned with in any final doctrine of development.

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## EXPLANATION OF PLATES

All figures made with the aid of Abbe camera lucida. Those of living eggs in outline only. Details supplied free hand. No attempt has been made to give exact magnification of living eggs, the erratic shapes making this extremely difficult.

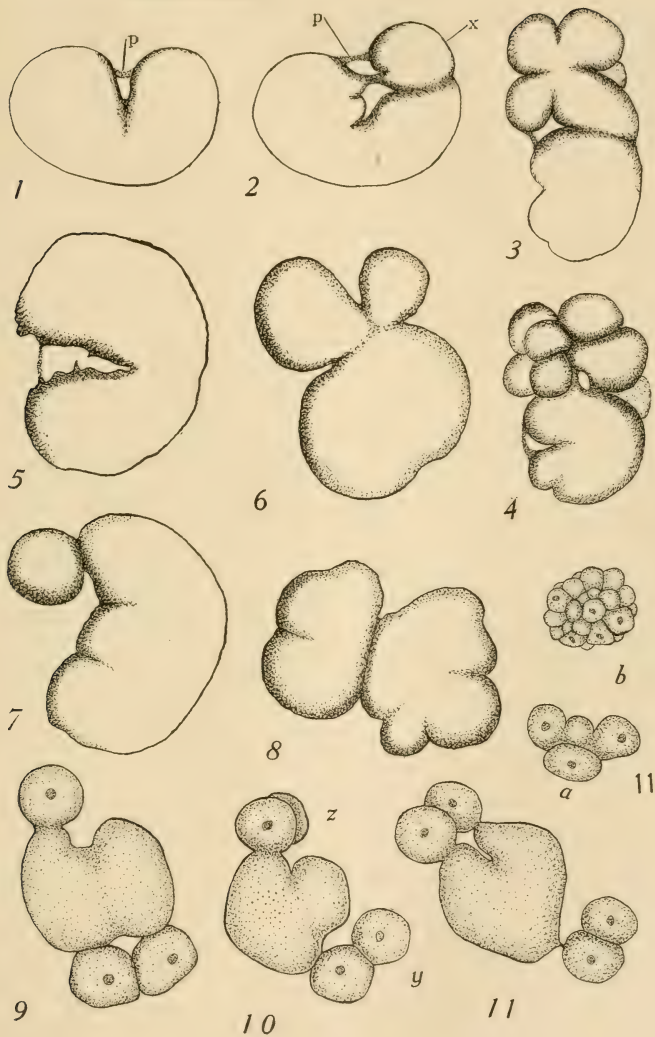
### PLATE I

#### EXPLANATION OF FIGURES

1 to 4 *Pennaria tiarella*. Varying aspects of cleavage in early phases, as a basis for comparing that of *Pennaria australis*. *p.* protoplasmic connective or strand; a very common feature in these eggs. *x*, a blastomere of second cleavage. In fig. 3 it will be noted that this blastomere segments more rapidly than the lower. This is very common, and continues in fig. 4.

5 to 8 *Pennaria australis*. Cleavage here resembles in a marked degree that of the preceding species.

9 to 11b *Hydractinia echinata*. An extremely erratic cleavage. In fig. 10 are shown several interesting features, viz. the blastomeres at *x*, *y*, *z*. At fig. 11 they are shown in a later stage, in which *x* and *y* are just becoming detached. Their later history is shown in figs. 11, *a* and *b*.



## PLATE 2

### EXPLANATION OF FIGURES

12 to 22 Various phases of cleavage of similar eggs. Figs. 14 to 18 phases of cleavage in a single egg at intervals of ten minutes. The irregular spaces shown are interesting as so-called segmentation cavities. As a matter of fact, they are but aspects of the peculiar ectosarcal and amoeboid activities, and hence absolutely devoid of any blastocoel relations. Such is likewise the case with other similar features in other cases.

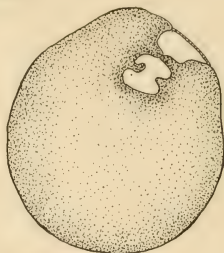
19 to 22 Varying phases in the cleavage of another egg, sketched at intervals of fifteen minutes. In these are shown in typical aspects the ectosarcal features more or less common in these eggs. The various strands, papillae, etc. are conspicuous.

12 to 13 Aspects of later cleavage of the egg body shown in figs. 9-11.

23 Gonophore of *Clava* showing the unusual feature of two perfectly formed and typical germinal vesicles in a single egg.  $\times$  about 100.



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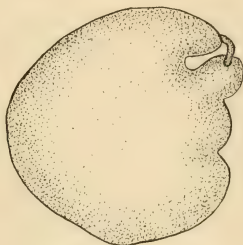
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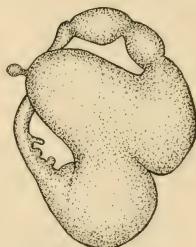
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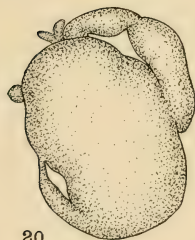
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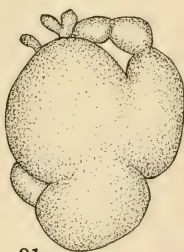
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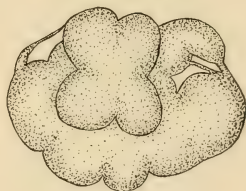
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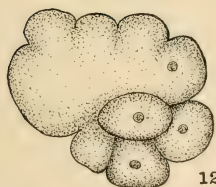
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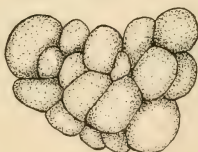
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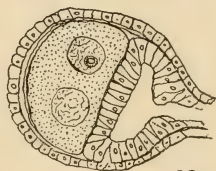
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## PLATE 3

### EXPLANATION OF FIGURES

24 to 27 Cleavage aspects of *Tubularia crocea*. (Reproduced from drawings made by G. T. Hargitt illustrating his paper on early development of *Pennaria tiarella* and *Tubularia crocea*. Bull. Mus. Comp. Zoöl., vol. 53, no. 3, by permission.)

24 Cleavage planes which are complete, are more or less vertical, but the equatorial furrows are shown in several of the blastomeres.

25 to 26 Two sections of an egg showing extremely elongated and erratic aspects. The several spaces shown are designated as cleavage cavities. This view I have taken occasion to question in the text of the present paper.

27 So-called blastula stage. This point I have also shown to be a mistaken view. In fact it may be questioned if in any case the term blastula should be applied to early stages of cleavage such as this.

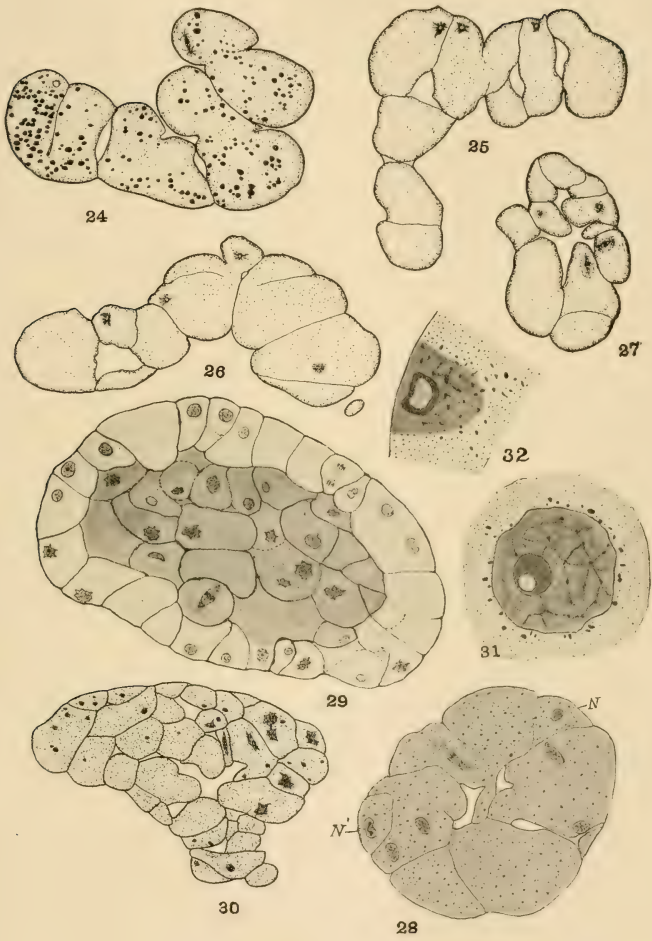
28 Section of an egg of *Pennaria tiarella* in early cleavage. This is an egg which has shown an unusual regularity in cleavage behavior. *N* shows a typical resting nucleus, of which several others are shown. At *N'* is shown a nucleus in what seems amitotic cleavage. In this egg are seen also several inner spaces, but which are extremely transient phases.

29 Morula of *Clava*. The pro-entoderm has been tinted to show the early physiological differentiation of these cells. A discussion of this may be found in the text.

30 Morula of *Hybocodon prolifer*. As compared with preceding figures of *Tubularia*, *Pennaria*, *et al.*, it shows the same indefinite intercellular spaces, but no distinctive blastocoel. Several nuclei here shown resemble much those of *Clava*, and appear in some cases in amitotic division.

31 Nucleus of *Clava* just prior to maturation. The nucleolus is conspicuously vacuolated. Chromatin is in process of fragmentation and dispersal. Stained by picro-hematoxylin which differentiates the yolk beyond mistake, and makes certain the chromatin nature of these granules.  $\times 800$ .

32 Nucleus of *Clava* in process of fragmentation and dissolution. First polar body already discharged. Nucleolus in process of collapse; chromatin fragmentation well advanced. Stain as in previous egg.  $\times 800$ .





# PHYSIOLOGICAL ANIMAL GEOGRAPHY

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NINETEEN FIGURES

## CONTENTS

I	Introduction.....	552
1	Faunistic animal geography.....	553
2	Physiological animal geography.....	554
II	The physiological characters and distribution of particular species of tiger beetles.....	556
A	Material: general habits.....	556
1	Reproductive processes.....	557
2	Larva and pupa.....	557
3	Food.....	558
B	Habitat relations of soil-inhabiting tiger beetles.....	559
a	C. <i>purpurea limbalis</i> .....	559
1	General behavior of adults.....	559
2	Ecological relations of adults.....	560
3	Ecological relations of larvae.....	561
4	Experimental studies of habitat selection.....	566
5	Geographic distribution.....	567
6	Geographic variation in habits.....	568
b	<i>Cicindela tranquebarica</i> .....	568
c	<i>C. sexguttata</i> .....	575
d	Other species.....	584
C	General considerations.....	586
1	Importance of breeding instincts and breeding place.....	587
2	Relation of behavior to the habitat and associated forms.....	588
3	Meaning of variation in habits.....	589
4	The relation of geographic to local distribution-governing factors.....	590
III	The physiological characters and distribution of groups of species (formations).....	591
A	Zoological opinions and difficulties.....	591
B	Nature of the environment.....	592
C	Environmental relations of animals.....	592
1	Comparison of the environmental phenomena of plants and animals.....	592
2	The most important relations of animals.....	595
a	The method of investigation.....	595

3	The relation of physiological characters to geographic range . . .	596
a	Laws governing the reactions of animals . . . . .	597
b	Law of minimum . . . . .	597
c	Law of toleration of physical factors . . . . .	598
4	Tentative laws of distribution . . . . .	600
D	Classification of environments . . . . .	600
1	Elementary principles of classification . . . . .	601
2	The best index of geographic complexes . . . . .	601
E	The animal formations . . . . .	602
1	Classification of formations . . . . .	603
a	Principles of classification . . . . .	603
b	The geographic formations of the world . . . . .	604
IV	The problems, methods and relations of physiological animal geography .	607
A	Problems . . . . .	607
1	Behavior . . . . .	607
2	Physiology . . . . .	608
B	Methods . . . . .	609
C	Relations to other subjects . . . . .	609
D	The future biology . . . . .	611
V	General summary . . . . .	612
	Acknowledgments . . . . .	613
	Bibliography . . . . .	615

## I. INTRODUCTION

Only a working knowledge of the facts of animal geography is necessary for the recognition of at least two or three lines for the development of investigation, and for organization into a science. Likewise, a casual inspection of the existing literature, indicates clearly that only one or at most two of the possible lines of investigation have received attention; facts have been accumulated very largely from the point of view of animal structure, and organization has been based on evolution. Physiological lines have been proportionately neglected. It is our purpose to point out some of the possibilities of investigation and organization along physiological lines.<sup>1</sup>

<sup>1</sup> When my paper on the life-histories and larval habits of the tiger beetles ('08) was prepared, it was my intention to follow it a year later with one on their ecology and distribution. While attempting to prepare this, it became evident that many of our so-called principles of distribution and some of the methods employed in its study, were not wholly trustworthy as a basis for generalization. Before interpreting the beetle data, further observation and the examination of the existing literature seemed advisable. We present here a point of view which developed in connection with this uncompleted task.

The materials of animal geography may be roughly classified into fact and interpretation. Interpretations have been related to genetic or historical geography, fields in which speculation has been common. Since the methods employed and the conclusions reached are quite familiar, we will take up the discussion so far as possible, from the point of view of facts.

The facts of animal geography fall under two main heads: (a) facts concerning the structural and the taxonomic differences and resemblances of the animals of different parts of the world, and (b) facts concerning the physiological and ecological characters of animals which enable them to live under the geographic conditions in which they are found, and the effect of geographic environments upon their behavior, physiology, and mode of life. The former is what is commonly known as faunistic animal geography, the field in which nearly all the investigation has been concentrated.

## 1. FAUNISTIC ANIMAL GEOGRAPHY

*a. Point of view.* The point of view is essentially that of speculative evolution, of the evolution of animal groups and of the evolution of barriers and land masses as related to the distribution and dispersal of animals from the supposed centers of origin (Wallace, '76; Osborn, '02). The subject is even more strongly committed to speculative evolution than any other phase of biological science.

The study of so many phases of biology from the point of view of an inadequate conception of evolution, which has been so prevalent during the past forty or fifty years, has probably materially retarded the unification and progress of biological science as a whole. In the case of the geographic aspect, the damage done is quite beyond repair, for the great mass of ecological data accumulated during that period has not been preserved and the conditions which make such observations possible have passed away, too frequently forever, before the hand of civilization (Haddon, '03; Webb, '03).

b. *History.* The history (Ortmann, '96) of faunistic animal geography is largely that of the ideas of regions, centers of dispersal, barriers, etc. The only recent writer who has advanced other ideas is Seitz ('91), who called attention to the resemblances of the forms of similar habitats in different parts of the world (Beddard, '95).

## 2. PHYSIOLOGICAL ANIMAL GEOGRAPHY

a. *Point of view.* There are two distinct points of view for biological investigation. One is that of *evolution*; the other, that of *physiology*, or the explanation of the organism in terms of physics and chemistry. One may make a physiological explanation of the behavior or structure of an organism and in no wise explain its evolution. On the other hand one may make an evolutionary explanation of an organism without making any contribution to its physiology. The study of physiological animal geography may be conducted independently of the problems of evolution. It does not need to be concerned with centers of origin, or paths of dispersal, or with other problems of faunistic animal geography. In this paper we are concerned solely with the *physiological relations of animals to natural environments*.

b. *History.* Crude expression of some of the ideas which should be included in this subject is no doubt as old as biology itself. From the standpoint of particular taxonomic groups, various writers on natural history, ecology, behavior and physiology have from time to time touched upon the relation of habits, ecology, or physiology to geographic distribution of particular species (Semper, '81).<sup>2</sup> Ortmann ('07) especially emphasizes the importance of a knowledge of the habits as a means of interpreting distribution. Indeed, this is an important principle of ecology, and lacks definite formulation rather than recognition.

From the point of view of all the animals of a given set of environmental conditions, Thomson gives the early history in his introduction to Brehm ('96). The early observations were made by men whom Thomson designates as naturalist travelers.

<sup>2</sup> Semper brought a large number of these facts together as they existed in 1879.

of the biological type, the most noted of whom were contemporaries of Darwin, such as Bates, Belt, Wallace and Brehm. Though anthropomorphic, at least in his wording, Brehm stands as one of the foremost writers of the time in this field of animal behavior.

He had unusual power as an observer of the habits of animals. His particular excellence is his power of observing and picturing animal life as it is lived in nature, without taking account of which biology is a mockery and any theory of evolution a one-sided dogma. The success of the pictures which Brehm has given us of bird-bergs and tundras, of steppes and desert, of river fauna and tropical forest, raises the wish that they had been complete enough to embrace the whole world. Thomson.

An excellent discussion by Craig ('08) who compares the behavior and adaptation of the birds and mammals of the steppe of North America with those of the forest,<sup>3</sup> is the only recent paper of this kind, by a zoologist, which has come to my attention. There has been, so far as I have found, no comparison of the behavior of the animals of the different deserts or different steppes, etc.

While physiological animal geography is a subject for experimental study, experimental methods can hardly be said to have been used in the study of geographic distribution. Experimental researches which have involved distribution are limited chiefly to investigations of the reactions and local distribution of aquatic and cave animals (Banta, '10).

In the field of plant geography, Schimper's ('03) work indicates the first step in the development of the world-wide aspect along physiological lines (Cowles, '09). This work opened a new and fruitful field for experimental work and field observation. Here Warming ('09), Cowles ('01), Whitford ('01), Transeau ('03, '05) and others contributed much from the observational side, while others have done important experimental work.

In the presentation of data and in the discussions here we illustrate two points of view for investigation by classifying the materials roughly into (*a*) those related primarily to the par-

<sup>3</sup> The work of Adams ('05, '09), and Ruthven ('06), was conducted with reference to all the animals but from a genetic rather than a physiological point of view.

ticular species of tiger beetles, and (b) those related to the entire group of animals inhabiting a given environmental complex.

## II. THE PHYSIOLOGICAL CHARACTERS AND DISTRIBUTION OF PARTICULAR SPECIES OF TIGER BEETLES

### A. MATERIAL: GENERAL HABITS

The tiger beetles are graceful, predatory, swift-flying insects, whose bright colors and great variability have long been familiar.

The following general account of habits applies to all the species especially considered here. The life-histories consist of the egg, three larval stages, the pupa and the adult. When the beetles

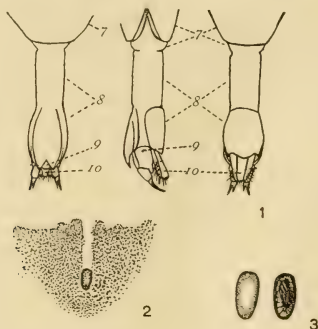


Fig. 1 From left to right—the ventral, side, and dorsal view of the ovipositor of *Cicindela purpurea* with segments numbered. Three times natural size.

Fig. 2 The egg of *C. purpurea* in position in the hole in the ground made by the ovipositor. One and one-half times natural size.

Fig. 3 The egg. Three and one-half times natural size.

emerge from the pupal stage in summer, they are not sexually mature. Many species hibernate during the winter following emergence. Hibernating species (Shelford, '08), reach sexual maturity after several warm days of spring. Previous to sexual maturity, the animals are in a different physiological state than when sexually mature, and they accordingly behave differently, congregate in different places, and never attempt to use the ovipositor.

### 1. Copulation and egg-laying

The beetles copulate on warm days, especially when the atmospheric humidity is high. The eggs are laid in small vertical holes, 7 to 10 mm. deep, made by the ovipositor (figs. 1, 2 and 3).

### 2. The larva and pupa

The larva, which on hatching excavates a vertical, cylindrical burrow in the position of the ovipositor hole, is elongated, yellowish, and grub-like, with a number of brown spots on each abdominal segment, and with a dark-colored, strongly chitinized head and prothorax of unusual form. The head bears two pairs of large ocelli on the outer border of the upper surface, two pairs of small ones on the lower surface immediately below them (figs. 4 and 5). The mandibles, instead of extending downward or for-

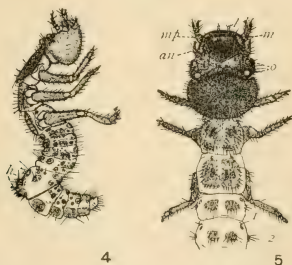


Fig. 4 The larva, side view; *h*, hooks. Three times natural size.

Fig. 5 The anterior half of the larva; *an*, antennae; *mp*, maxillary palps; *m*, mandible; *o*, ocelli. Three times natural size.

ward as is usual in insects, are curved upward, and when closed, overlap above the anterior end of the clypeus. The lower side of the head is somewhat hemispherical, the upper side flattened, and, with the appendages, almost semicircular in outline. The prothorax is semicircular, flattened above, and projects at the sides. Taken together, the head and prothorax form nearly a circle. The meso- and metathorax and abdomen are soft and fleshy. On the dorsal side of the fifth abdominal segment is a

hump-like outgrowth which bears a pair of long, curved, anteriorly directed hooks (*h*, fig. 4), a pair of short vertical spines, and many strong bristles. The last two abdominal segments are also armed with strong bristles.

In moving up and down in the burrow the larva uses the dorsal hump, the legs, and the last abdominal segments. The animal turns around in the burrow by bending the anterior part of the body dorsally, and forcing the head past the dorsal side of the abdomen which is held in position while the anterior part is moved by means of the feet. When at rest in the burrow, the animal assumes a zigzag (Enoch, '03) position as shown in fig. 4. When waiting for prey at the mouth of the burrow, the same general position is maintained, but the head and prothorax are bent at right angles to the longitudinal axis of the meso-metathorax. The legs, the vertical spines of the dorsal hump, and the strong bristles of the last two segments hold the animal in position. The head and prothorax just close the round opening and the mandibles are extended. If a small or medium sized insect pass near, the larva strikes at it with its head, by suddenly straightening the body in the region of the meso- and metathorax (Geoffroy, 1762), at the same instant closing the mandibles with a snap that can be distinctly heard, if the prey escapes them. If the insect caught be of small size, the larva darts backward to the bottom of the burrow with its prey which is devoured at leisure, the inedible parts being brought to the surface and cast out. If the prey be large (for example, a cabbage butterfly, as was observed by Weed, '97), it is held at the entrance of the burrow. The forward projecting hooks of the dorsal hump serve to prevent the butterfly from dragging the larva out of its hole, while its blood is being withdrawn. The pupa is of the usual beetle type (fig. 6). Pupation takes place in the ground.

### 3. Food

The food of both larvae and adults consists of sow-bugs, centipedes, spiders, dragon-flies, butterflies, beetles, flies, and larvae of all sorts, in fact, any small animals that come within

reach. If larvae are not fed, they will not die for a week or two, or even longer, but the lengths of their periods of growth are greatly increased.

B. HABITAT RELATIONS OF THE SOIL-INHABITING TIGER BEETLES  
(CHARACTERISTIC DATA)

The environmental relations will be illustrated by the relations of three species of *Cicindela purpurea* Oliv. subspecies *limbalis* Klg., *tranquebarica* Herbst, and *sexguttata* Fabr.



Fig. 6 The pupa. Three times natural size.

a. *Cicindela purpurea limbalis*

The adults are beautiful red and green, though not strikingly conspicuous forms. Eggs are laid in June; the larvae hibernate usually in the second instar and pupate in the second summer. The imagoes emerge about a month after pupation, hibernate, and become sexually mature late in the third June. The larval life lasts twelve to thirteen months; adult life, ten months; two years between generations.

1. *General behavior of adults.* They are not strong fliers, but are very alert and start to fly whenever one approaches them. The form of the moving object is not important; size and movement produce the reaction apparently without reference to form and color. I have not been able to ascertain that they turn and face an approaching person with any degree of uniformity,

as is asserted by Comstock ('04), and have never seen them fly into vegetation, or crawl into crevices.

2. *Ecological relations of adults.* a. General conditions at the point of study. My studies have been conducted along the west shore of Lake Michigan between Lake Bluff and Winnetka,

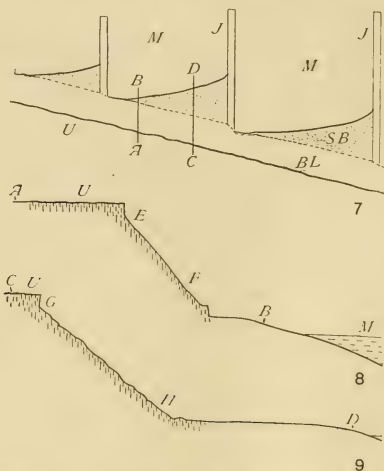


Fig. 7 Diagram showing Lake Michigan bluff as seen from the zenith. *U*, level surface of upland; *BL*, bluff; *SB*, sandy beach; *M*, water, *L. Mich.*; *J*, piers; toward the left is north; sand has lodged on the north side of the piers. *AB* and *CD* indicate positions of cross-sections below.

Fig. 8 Cross-section *AB*. Slumping bluff stage. The adults of *C. limbalis* are distributed from *A*—*B*; the larvae, sparingly, from *E* to *F*. Other letters as in fig. 7.

Fig. 9 Cross-section *CD*; stage of some bluff stability and bare clay exposure. Adults of *limbalis* between *C* and *D*; larvae plentiful between *G* and *H*. Other letters as in fig. 7.

Illinois, but my attention has been concentrated on the habitats near Glencoe, Illinois.

Between the points mentioned, the lake is eroding its morainic shores. Steep banks have been formed by this action which are from 11.4 meters (38 feet) to 20.4 meters (68 feet) in height.

The steepness of the slope makes conditions severe for plant life. It is only where inactivity of forces of erosion has decreased the steepness of the slope, that scattered plants are present. Where the slope is still less steep, the bluff is sometimes covered with forest.

On the upland adjoining the bluff are stretches of meadow, woods, and sometimes pastures, all intersected by paths, roads, and ravines. All these furnish bare ground which apparently is essential to these tiger beetles. At the base of the cliff is frequently found a narrow stretch of sandy beach, which varies in width from 1 to 25 meters (figs. 7, 8 and 9).

b. Local distribution. The adult beetles of *C. limbalis* are found on the upland near the bluff in all of the bare places just



Fig. 10 The burrow of *C. purpurea limbalis*; *p.c.*, pupal cell. One-third natural size.

described, and on the steep clay bank and the sandy beach—about equally distributed in proportion to the area of the bare soil exposed (figs. 7, 8, 9). If the number be greater in any one of the situations, it is on the sandy beach. If the adults be about equally distributed on the different areas, which of these are we to consider the habitat of the species? Let us inquire into the habits of the larvae.

3. *Ecological relations of the larvae.* a. Local distribution. I have carefully watched the larvae of this species (fig. 10) in their external environmental relations for five years in the vicinity of Glencoe. They are found almost exclusively on the clay bank (fig. 11). Occasionally larvae are found in bare places on the steep banks of the ravines. Three or four individuals were once found on the top of the bluff in a bare place on level ground,

## EXPLANATION OF FIGURES

11 The habitat of *C. purpurea limbalis* near Glencoe, Illinois, showing several stages in the development of the forest on the bluff. The area to the right of the imaginary line between *a* and *b* is stable enough to support some sweet clover. Here the tiger beetle larvae are most abundant. The area between lines joining *a* and *b* and *a* and *c* is in the early shrub stage. To the left of *a c* the shrubs are denser, and larger and some trees are present.

12 Habitat of *C. tranquebarica* in the pine zone of the ridges at the south end of Lake Michigan. The dark portion in the foreground is the shadow of a tree. At the left is the cattail zone of the depression; between *a* and *b*, the sedge zone; between *b* and *c* the zone of high depression plants. The white blossoms here are those of *Parnassia caroliniana*; their distribution, September, 1906, corresponds approximately to the distribution of the larvae of *C. tranquebarica* which arose from eggs laid in May and June, 1905. The portion above and to the right of *c* represents the higher portion of the ridge and the habitat of *C. scutellaris*.



but these are the only ones found in such a situation, as compared with over six hundred actually dug from the clay bank. They are entirely absent from the sandy stretch at the base of the bluff. I have dug very many larvae of *Cicindela lepida* from this situation, and have never found a single larva of *C. limbalis*. However, larvae of *C. limbalis* are not equally abundant on all parts of the clay bluff. The portions which are very steep, subject to land slides in the spring, and very dry in summer, are essentially without larvae. The forest covered portions are without larvae. The shrubby parts are inhabited only in the open places. The bare places with a few herbaceous plants have the *greatest number of larvae*.

b. Migration of larvae. As I pointed out in 1908, the larvae of this species rarely migrate, but remain at the point where the egg was laid. Only fifteen per cent of them left their burrows during a period of two or three weeks after they had been dug from their normal habitat and placed in holes made with a wire in moist sand. In eighty-five per cent of the cases the larvae smoothed off the sides of these burrows, and remained in this very unnatural situation—one in which all of the physical conditions had been changed. The steep, sloping clay had been replaced by level sand, resistantly packed particles of clay, by coarse sand grains, and the solid edge of the burrow (fig. 10) by the crumbling sand. In the field, I have never seen larvae crawling on the ground. Burrows have been found empty in a few cases in digging about six hundred larvae. In one or two cases the dead larvae were found in the burrows and as these would soon disintegrate and leave the burrow apparently empty, vacant holes may have been left in this way. Then again, ants may overcome a larva, and after chewing off its antennae and tarsal joints, drag it from the burrow. While larvae may occasionally migrate, the empty holes are not so numerous but that their occurrence may be due to other causes.

c. Local distribution of larvae dependent upon adjustment in egg-laying. The larvae vary in position from year to year apparently with the weather conditions at the time of egg-laying. They live for a little more than a year. In 1906 the full grown larvae

were found on the higher and drier parts of the clay bank. The eggs from which these larvae were hatched were laid in June of 1905. The total rainfall at Chicago, from January to June inclusive, was 42.5 cm. (17.1 inches), for April, May and June 29.0 cm. (11.5 inches), and for May and June 21.0 cm. (8.4 inches) and for June 8.0 cm. (3.2 inches).

In 1907, on the other hand, they were on the low places near the springy situations and in small gullies, the eggs from which these larvae hatched having been laid in June 1906. The rainfall from January to June inclusive in 1906 was 29.0 cm. (11.6 inches), from April to June inclusive 14.5 cm. (5.8 inches), for May and June 10.0 cm. (4.0 inches), and for June 4.7 cm. (1.9 inches).

The failure of the larvae to migrate stands out clearly even a year after the egg-laying took place. The larvae of this species usually adjust the depth of their burrows to the temperature conditions of the sand in which they were placed under experimental conditions. In nature, however, I doubt that these larvae can dig their holes deeper when the soil becomes dry and the temperature high, because at such a time the clay is very hard.

d. Relation of larval habitat and distribution of food to the distribution of adults. In a natural indentation of the coast at Lake Bluff, Illinois, a beach of considerable width has been deposited and the bluff bears a very dense forest. No larval habitat is present, and the adults of *C. limbalis* are not present on the beach. Their food is at least as abundant here as where the clay bank is bare.

As we have stated, the tiger beetles feed over an area much more diversified and much greater in extent than the breeding place or larval habitat.

The adult beetles feed on any available animals. The feeding areas which are adjacent to like breeding places differently located, are frequently very different, and are occupied by very different food species. The food is then of necessity different for forms living in different places.

In captivity the adults have been fed with lean meat of various sorts (beef, pork and mutton) which they eat readily when fresh. They also pick up the ants, Thysanurans, etc., in the cages, and

when not fed, devour their own species. The food relations are, then, highly regulatory, the animal feeding on available food.

4. *Experimental studies of habitat selection.* a. Methods of experimentation.<sup>4</sup> Do the adults select their egg-laying place? To answer this question, adults were placed in cages containing soil of several kinds. Each kind was so arranged into steep and level parts, that about one square foot of each type was exposed. The adults placed in the cage were taken when the species was copulating freely. The soil was kept very moist up to the time the first ovipositor holes were made because this species lays only in moist soil. After this the wetting of the soil must be done very cautiously, so as to prevent washing eggs from the ground in steep parts. Accordingly, the holes were not obliterated from day to day and the counts are not accurate for the soil in which a large number were made because eggs are sometimes laid very close together and adjoining holes destroyed. Some eggs are deposited in irregular cracks and crevices where they are likely to be overlooked. The greatest care was taken to discover every hole made in the soils in which larvae do not occur in nature.

b. Results. The following table shows the approximate number of holes made in the clay and probably the actual number

TABLE 1

*The distribution of ovipositor holes and larvae of C. purpurea limbalis under experimental conditions*

		CLAY		CLAY, 9 PTS. HUMUS, 1 PT.		FOREST HUMUS		HUMUS, 1 PT. SAND, 9 PTS.		CLEAN SAND	
		S	L	S	L	S	L	S	L	S	L
Lot I.....	Holes.....	0	0	0	0	0	0	0	0	0	0
	Larvae.....	9	0	0	0	0	0	0	0	0	0
Lot II.....	Holes.....	21	5	0	0	0	0	0	0	0	0
	Larvae.....	12	1	0	0	0	0	0	0	0	0
Lot III.....	Holes.....	17+	7+	1	0	0	0	0	0	0	0
	Larvae.....	24	10	1	0	0	0	0	0	0	0

S = steep; L = level.

<sup>4</sup> Each experiment requires daily attention for from one to two months, as well as considerable greenhouse space.

made in the other soils, together with the number of larvae which appeared; 80 per cent on the steep slope, 98 per cent in clay.

The count of holes includes some in the first stages of digging, mere scratches on the ground, and others which had been excavated to the usual depth with or without eggs being laid.

c. Factors controlling egg-laying. Pairs taken in coitus were placed in cages containing sand only and level clay only. No larvae appeared in either case. The experiment with the level clay has not been repeated. Females placed in cages containing rough, steep clay, deposited eggs. Eggs are also absent from dry soils, whether steep or level.

Slope, kind of soil and soil moisture are factors governing the deficiency or absence of eggs. A deficiency or excess in any one of these respects decreases the number of eggs laid, or causes them not to be laid at all. The animals are in the condition for egg laying for a short period.

d. Method of selection. It has been determined by opening holes that eggs are not laid in all, and in one case the first holes made by a female were empty. This would tend to show that they try the soil before laying the eggs, but I have not been able in other cases to determine whether the first holes contained eggs or not. To determine this, it would be necessary to watch a female all of the time during several days.

e. *Geographic distribution.* a. Distribution of the species. This species occurs from the Island of Mount Desert on the coast of Maine, northward along the coast of Nova Scotia and New Brunswick, up the St. Lawrence River, through the region of the Great Lakes, and westward across the northern part of the great plains to Alberta, and south along the eastern slope of the mountains; southward in the upper Mississippi Valley to St. Louis. Its place in the great plains and southern prairie region is taken by other forms recognized as other color varieties, but so far as is known, similar in habits. These forms are the varieties or subspecies *transversa*, *splendida*, *amoena*, *denverensis*, and *ludoviciana*. *Splendida* has recently been recorded by Sherman from western North Carolina. Occasional specimens are recorded from Kentucky, Tennessee, and northern New Jersey. It is evident,

since none of these states were mentioned in the old state records of early collectors, that the beetles have dispersed into this region with the cutting of the timber and the building of roads and railroads, which have exposed large areas of clay bank.

There are also taxonomic difficulties and lack of knowledge of larval habits. No map of the distribution of the species will be published until we have investigated the subject further. It is clear, however, that the distribution area of *limbalis* is one in which moist clay banks are common. It represents the margin of the ice sheet, the region of extensive clay deposits which are being eroded rapidly, and the slope of the mountains where erosion is also rapid. It is closely correlated with the behavior of the species. Its geographic distribution appears to be determined by the same factors as its local distribution.

6. *Geographic variation in habits.* The relations to soil and topography do not vary greatly geographically. The various races mentioned as occurring in the southern part of the range of the series differ sufficiently in structure and color to constitute subspecies in the opinion of good taxonomists. Still a number of observers, Messrs. Lantz, Wickham, Wolcott, Smyth, and Cloverdale, tell me that the adults of all are associated with clay banks.

Near Chicago the larval life is a little more than a year, thirteen to fourteen months, and the adult life ten to eleven months. Criddle ('10) has confirmed his statement ('07) that the larval life lasts two years in Manitoba. The depth of larval burrows in Manitoba is 15 cm., near Chicago, Illinois, 5-10 cm.; the adult burrows at Aweme are 15 cm.; at Chicago, in captivity, 5-8 cm.

#### b. *Cicindela tranquebarica* Herbst

The usual color of the adults in eastern North America is brown. The life-history differs from that of *C. limbalis* in the following points: (1) Eggs are laid in May; (2) larvae pass the winter in third stage.

1. *General behavior of adults.* They are a little shyer than *C. limbalis* and more difficult to capture. They start when approached by a moving object, and when alighting, frequently

turn toward the observer. They almost never alight on vegetation. When caused to fly up from a narrow path, they frequently fly in a circle and return to a point behind a person moving forward. I have never seen them crawl under objects when pursued. They excavate burrows for the night and cloudy days.

2. *Ecological relations.* a. Area of special study. They have been studied specially at the south end of Lake Michigan. Here the species is found only on the ridges with pines. These ridges were originally thrown up under water near the shore. By the falling of the surface of the lake, which has amounted to a total of 18 meters since glacial times, ridges have been left out of water perhaps about as fast as they were formed. We have, then, a series of them of different ages, arranged in order of age. The youngest are nearest to the shore. Their width varies from five to thirty meters. Long, narrow ponds of corresponding age occur between the ridges. As a given ridge came above the surface of the water, it often received wind-blown sand; there is little or no vegetation on the youngest ridges.

b. Local distribution. *C. tranquebarica* is absent from the ridges with sparse vegetation. On the ridges on which young conifers are found together with various herbaceous plants along the pond margins, *C. tranquebarica* is present. Adults are numerous along the margins of the ponds and all over the ridges, particularly on the sandy 'blowouts,' or points where the wind has removed some of the sand and keeps the vegetation from growing up. The beetles frequently burrow into the sand for the night and for hibernation. Food is abundant on the white sand areas and the beetles find advantage in its conspicuousness, which no doubt causes them to congregate on these places to feed.

When an area of denuded sand, in which ponds or depressions are present, is deposited or exposed, vegetation appears first nearest the water. Humus accumulates, blackening the soil and making conditions favorable for more plants, so that a turf is soon formed near the water. Similar processes are going on higher up on the side of the pond margin and it is soon captured by the plants. It is on the ridges in which the soil just above the very moist or sedge zone is blackened by humus, but still not completely occupied by the roots of plants, that we find *C. tranquebarica*.

The succession of plants does not end here, and we find shrubs coming in and the turf migrating farther and farther up the slope of the pond margin. Shrubs shade the pond margin. The pines on the ridges are displaced by oaks and the undergrowth of herbaceous plants becomes denser; the pond margins are densely covered with turf or shaded by shrubs and trees. Though the higher portions of the ridges, namely, the feeding grounds, are still bare, *C. tranquebarica* is not to be found. The species must then have some vital relations to the pond margin.

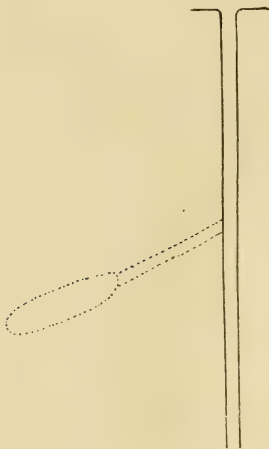


Fig. 13 The upper part of the burrow of *C. tranquebarica*, pupal cell shown by dotted line. One-third natural size.

3. *Ecological relations of the larvae.* a. Local distribution. The general behavior of the larvae is similar to that of *C. limbalis*. The holes are, however, deeper and straight (fig. 13). The larvae of *C. tranquebarica* are found in clay, alluvium, or sand, and have been reared or identified from all of the kinds of soil mentioned in the discussion of the adults. In our sandy area of special study, they are found near the pond margins only. In all the localities referred to in connection with the adults, the larvae have

been found in soils with a moisture content similar to that near Chicago.

b. Migration of the larvae. The larvae of this species rarely migrate. I have watched the larvae that appeared in the experimental cages after the soil had been permitted to become very dry, but none of them moved during several weeks.

c. Variation in local distribution. The distribution of larvae in Gary in 1906 corresponded to that of the white blossoms of *Parnassia caroliniana* which are shown in fig. 12. Their position varies from year to year, according to the rainfall as in the case of *C. limbalis*.

d. Relation of the larval distribution to the distribution of the adults. On the pond margins where herbaceous plants have been displaced by shrubs, *C. tranquebarica* is not present, although the higher parts of the ridges are bare and much like they are where *C. tranquebarica* is present, indicating that the adults disappear with the larvae.

4. *Experimental studies of habitat selection* a. Method. Adults of *C. tranquebarica* were placed in cages which were much like those which were used in the work on *C. limbalis*, but the soil was all essentially level.

b. Results. The results were as follows:

TABLE 2

*The distribution of ovipositor holes and larvae of C. tranquebarica under experimental conditions*

		CLAY	HUMUS	SAND, 9 PTS. HUMUS, 1 FT.	CLEAN SAND	SAND, 1 FT. HUMUS, 1 FT.
1907	Holes.....	7	0	13+	19	wanting
	Larvae.....	4	0	25	1	wanting
	Holes.....	?	3	25+	18	wanting
	Larvae.....	11	3	31	1	wanting
1908	Holes.....	16	wanting	29+	11	46
	Larvae.....	5	wanting	41	7	24

c. Factors controlling egg-laying. One striking difference is that the females did not lay with the same precision as did the

females of *C. limbalis*. Very many holes were made in the fresh, clean sand, but eggs were laid in only a few of them. These holes in the fresh sand have frequently been opened and found to be without eggs. Why fresh, clean sand should be so attractive to the females and fail to satisfy the final act of egg-laying is strange. Pure humus appears to be avoided when either moist or dry.

During the experiments, the different kinds of soil were kept as nearly equally moistened as possible, but a slight depression was provided in each. These were wetter and were especially selected by the females when standing water was not present. Eggs are not laid in dry or very wet soil. Moisture is evidently an important factor in controlling the egg-laying. I have found the beetles copulating and depositing eggs in my cages, on damp, cloudy days. This has not been observed in the case of most other species. It would appear, then, that light is not very important. However, as in the case of *C. limbalis*, deficiency or excess in one factor is sufficient to cause the soil to be avoided or only little used.

5. *Geographic distribution.* The habitat relations of *C. tranquebarica* are less definite than those of *C. limbalis*. We have found it on the bare clay of the overflow flats of the Arkansas River at Dodge City, Kansas, depending on stream moisture; on a path at the top of a terminal moraine at Waverly, New York, depending on climatic moisture; on alluvium along the Des Plaines River at Lyons, Illinois; and on the residual and alluvial soils of various parts of Colorado, New Mexico, Nevada and Idaho. In nearly all these localities, the soils examined were similar in their moisture content. The species is always nearer water courses in the more arid climates. The only place in which the soil moisture was deficient about the burrows was at Las Vegas, Nevada, at the height of the dry season. This is a region of winter rain, where the soil would be much moister in spring, the egg-laying season of the species. The larvae were much nearer the water (Las Vegas Wash) than I have found them in the moister climates. The bottoms of the burrows were nearly as moist as we commonly find them near Chicago.

This species includes several races which seem, according to the accounts of entomologists and my own observation, to be very similar in habits. It stretches across the middle region of North America, and ranges from the sea level to 7536 feet and throughout four of the zones of Merriam without regard to vegetation, efficient temperature or other climatic condition (table 3). A consideration of the races involved is necessary (Horn, '05; Wickham, '06). The records represented by dots (fig. 14) adjoining the Pacific Coast are for well recognized races. All others have been cast into synonymy by good taxonomists. The remaining records including 1-9 of fig. 14 are then for a single race. Furthermore two of the races sometimes recognized, *horiconensis* and Wickham's southern race, have been produced by suitable conditions during the late larval and pupal life. Table 3 shows the relation of a single race to climatic conditions.

TABLE 3

*The relation of the distribution of C. tranquebarica to climate. Vegetation and rainfall are approximated, especially for Alberta and B. C. Life zones are approximated where detailed maps are not available. The vegetation at Kalso is in question but the species has been taken in the mountains near, where there can be little doubt that it is coniferous forest. The numbers at the left refer to fig. 14.*

PLACE	STATE	ALTITUDE IN FEET	MEAN RAIN FALL IN INCHES	VEGETATION	ZONE (MERRIAM)	COLLECTOR
1   Woods Hole	Mass.	5	45.0	deciduous forest	Transition	Author
2   Meridian	Miss.	358	58.0	deciduous forest	Lower Aus- tral	U. S. N. M.
3   Alamosa	Col.	7536	15.0	steppe	Upper "	Author
4   Aweme	Man.	1180	17.45	steppe	Boreal	Criddle
5   Innisfail	Alb.	3600	15.0	steppe	Boreal	T. N. Will- ing
6   Caliente	Nev.	4407	7.0	desert	Lower Aus- tral	Author
7   Hagerman	Id.	2600	10.0	semi-desert	Upper Aus- tral	Author
8   Kalso	B. C.	1870	25.0	conifer for- est	Boreal	I. W. Cockle
9   Bridgeport	Cal.	64	3.7	desert	Lower Aus- tral	Wickham



Fig. 14 The distribution of *C. tranquebarica* as shown by locality records. The map indicates general topography. The numbered localities are selected to show relations of the distribution of a single race to topography, climate, vegetation, and Merriam's zone (p. 573).

Such distribution is characteristic of species which occupy environments made by streams, lakes, soil, or other local conditions. Such species are local in their distribution.

6. *Geographic variation in habits.* The life-history at Chicago is similar to that of *C. limbalis*. Criddle expresses the opinion that the larval life is two years in Manitoba, but has not yet confirmed the statement. The depth of larva burrows at Chicago is 22-50 cm.; at Aweme, Manitoba, 43-50 cm.

*c. Cicindela sexguttata*

This is a brilliant green form. Its life-history differs from that of *C. limbalis* in the following points: (1) Egg laying occurs about one week later, (2) larvae pass the winter in both second and third stages, (3) the adults emerge in August, but usually remain in the pupal cells until spring.

1. *General behavior of adults.* The adults of this species are less alert than those of the other species just discussed. They frequently fly and alight on leaves of bushes. When frightened in the woods they frequently crawl under a leaf or other object on the ground. Sometimes they remain very quiet for a time when the body is not all covered and the bright green wing covers stand out in contrast to the brown leaf under which they are hiding.

They crawl under the bark of trees at night and in cool or cloudy weather, both in nature and in cages, and rarely dig into the soil. But one individual moved soil when in captivity. This one was in a cage in which a piece of bark lay on the sand present. It was found to have removed a small amount of sand to make room for its body under the bark.

2. *Ecological relations of adults.* *C. sexguttata* has been studied in Massachusetts, New York, Illinois, Indiana, and Tennessee. It lives only in or about forests of a certain particular type. It is entirely absent from those that have developed on low, wet ground, such as marsh forests and humid climate flood-plain forests; it is not found in the early stages of the oak forest nor in the beech and maple (the climax forest of eastern

North America) nor in the cotton wood or true coniferous forests. It is abundant in and about the white-oak, red-oak hickory forest (figs. 15 and 16).

Climatic conditions influence the relations of this species to different types of forests, e.g., in eastern Tennessee they are found in much more xerophytic forests than in the vicinity of Chicago where the rainfall is appreciably less.

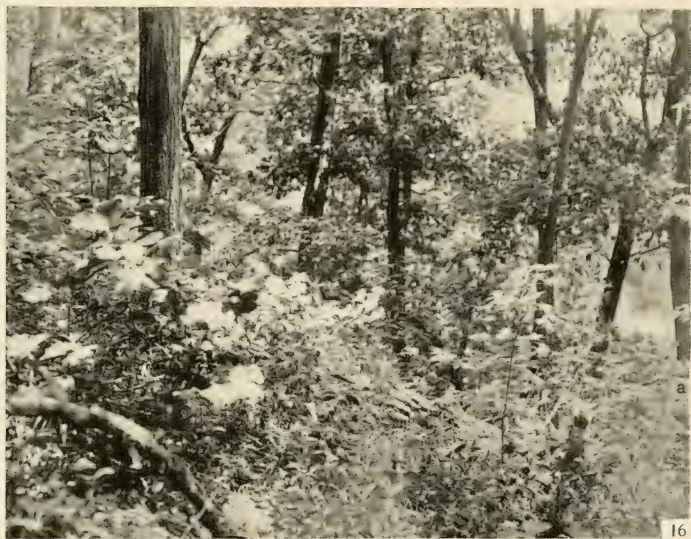
#### EXPLANATION OF FIGURES

15 General view in east Tennessee.

16 An open place in the oak and hickory forest of the mountain side, a typical *C. sexguttata* habitat. The individuals were seen copulating on the log in the foreground.



15



16

The Cumberland mountain district was originally completely forested. The forest of the valleys was chiefly beech and maple; of the mountain slopes, oak and hickory; of the mountain tops conifer. The soils are various, resulting from many different kinds of rock. We were unable to find this species in the strictly red cedar, pine or beech forest. However it occurs in the more mesophytic oak containing ravines of strictly conifer forests and in forest of mixed oak and conifer. Sherman records it from such situations also. This is not true near Chicago.

The beetles come out into the little streaks of sunshine on fallen trees and bare ground in the early forenoon to feed. The writer has seen them picking up insects from the logs in such locations. From my observations in the field I am confident that bare spots of mineral soil, fallen trees, etc., are essential to this species.

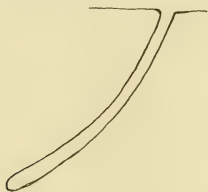


Fig. 17 The burrow of *sextguttata*. One-third natural size.

It is only in such places in virgin or little disturbed forests that I have found them copulating. However it is not a particular type of forest that is essential to this or any other species of tiger beetle, but a certain environmental complex in which a certain consistency and moisture of soil and a certain amount of sunlight and bare ground are the essential things.

3. *Ecological relations of larvae.* a. General behavior. The burrow resembles that of *C. limbalis* and is shown in fig. 17. This larva is less active than those of the other species, but otherwise is similar in habits.

b. Local distribution. The larvae of this species are very difficult to find because they are for the most part under leaves.

In eastern Tennessee I found them in bare spots on the steep mountain slopes where steepness of slope had prevented the accumulation of leaves, and in parts of the forest that had been fired recently and the leaves accordingly removed. They occur in clays resulting from the weathering of the following rocks: Briceville shale, Newman limestone, Knox dolomite, Chicamauga limestone, and Conasauga shale. Near Chicago they have been found on clay till, and on sandy till mixed with humus.

4. *Experimental studies of habitat selection.* a. Method. This species has been placed in cages containing various kinds of soil as have the others. The light relations were the same as in all of the other experiments, although the light is of more importance.

b. Result: Tables 4-8. Distribution of larvae of *C. sexgutata* under experimental conditions.

TABLE 4

*Relation to slope; sand dry at surface*

1907	CLEAN SAND		SAND, 9 PT. HUMUS 1 PT.		CLAY		CLAY, 9 PT. HUMUS, 1 PT.		FOREST HUMUS		REMARKS
	S	L	S	L	S	L	S	L	S	L	
Holes.....	0	0	0+	0	0+	0+	0+	0	0	0	S = steep L = level
Larvae.....	0	0	12	0	4	7	5	0	0	0	

TABLE 5

*Relation to shade; sand dry at surface. Sunlight in cages is reduced to one-third by glass roof and cage screen*

	CLEAN SAND		SAND, 9 PT. HUMUS, 1 PT.		CLAY		FOREST HUMUS		REMARKS
	S	U	S	U	S	U	S	U	
Holes.....	?	?	8	0	8	1	0	0	S = shaded U = unshaded
Larvae.....	0	0	8	0	0	0	0	0	

TABLE 6

*Clean sand, moist*

1908	CLEAN SAND	SAND, 9 PT. HUMUS, 1 PT.	CLAY	SAND, 1 PT. HUMUS, 1 PT.	ONE LEAF PLACED IN EACH CAGE
Lot 1					
Holes .....	5	5+	18	1	
Larvae .....	4	6	4	1	
Lot 2					
Holes .....	2+	0+	0+	0	Clay very dry
Larvae .....	4	3	1	0	
Lot 3					
Holes .....	0	10	10+	5	Two under leaf
Larvae .....	0	3	12	1	
Lot 4					
Holes .....	2+	11+	54	36	Three under leaf
Larvae .....	6	37	13	29	

TABLE 7

*Relation to thick covering of leaves*

	CLEAN SAND	SAND, 9 PT. HUMUS, 1 PT.	CLAY	SAND, 1 PT. HUMUS, 1 PT.	REMARKS
Larvae .....	7	34	28	8	{ All soil covered with leaves ex- cept clean sand

TABLE 8

*Total larvae shown in tables 6 and 7*

	SAND	SAND, 9 PT. HUMUS, 1 PT.	CLAY	SAND, 1 PT. HUMUS, 1 PT.	REMARKS
Larvae .....	21	90	56	43	Grand total 210

Total under leaves: 76.

c. Factors controlling egg-laying. The relations to light and leaves are interesting. The larvae were frequently at the edges of the piles of leaves in such positions as eggs might be placed by females, the posterior half of whose bodies were concealed by the leaves (fig. 18). Females have been found making ovipositor holes, when the posterior two-thirds of the body was under a leaf.

The depositing of eggs in the unshaded portions of the cages may be due to the reduced intensity of light, or the shadow of the cage frames, which falls upon some parts of the soil at any

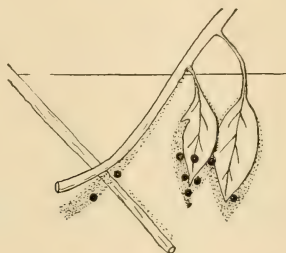


Fig. 18 Showing the position of the larvae of *C. sexguttata* in a cage. The black spots represent the larval holes. The stippled portion represents the approximate area in shadow during the middle of the day.

given time. Nearly all parts pass into shadow for a time during each day. All of the eggs may then have been deposited when all or a part of the animal's body was in the shade. The reduction of the intensity of the light to one third that of normal out-of-door light may act as a partial shadow to this species. The experiments will be repeated in the full sunlight.

As will be seen by an inspection of the tables most of the eggs were deposited in the sand with a little humus. None were laid in pure forest humus. The fresh sand and clay were ignored when they were allowed to remain dry. Muddy places were avoided. It is evident that egg-laying is governed by (a) kind of soil, (b) soil moisture, (c) slope, (d) light, (e) temperature (activity only

on warm days). Under conditions unsuitable in any one factor, few or no eggs are laid.

5. *Geographic distribution.* The geographic distribution of *C. sexguttata* is exactly what the general habitat relations would lead one to expect. Within fifty miles of Chicago, I have found it always associated with the white-oak and red-oak and with a single exception also the shag bark hickory. The same is true in east Tennessee. Comparing the distribution of the trees, we find that the combined extent of the white-oak and hickory represent almost exactly the distribution of this species (fig. 19). That is, the geographic distribution is the exact function of the local distribution (Ruthven, '07).

Fig. 19 A combination of the maps of Schimper '03, and Transeau, '03, showing the geographic plant formations of North and northern South America and the distribution of *Cicindela sexguttata*.

1a, c, d are forests with broad thin leaves.

1a. Dense tropical evergreen forest, rain-forest.

1c. Dense temperate evergreen forest, temperate rain-forest.

1d. Deciduous forest. The large black dots in this area represent locality records of *C. sexguttata*; the heaviest dots combined with crosses are placed over the centers of states from which it is recorded. The lines *x* and *y* show the relation of its distribution to that of two characteristic trees of the deciduous forest. The continuous line (*x*) represents the distribution limits (except along the Atlantic Coast of the white-oak (*Quercus alba*); the broken line (*y*) represents the distribution limits of the shag bark hickory (*Hicoria alba*), except where its limits are coincident with those of the white-oak. The distribution of these is not the same as that of the deciduous forest because the map is based on area with more than twenty per cent of woodland. In the savanna region (3b) these trees occur along the streams as does *C. sexguttata*.

2. Coniferous forest (with narrow thick leaves).

This is mixed with the deciduous forest in the region of the Great Lakes. In southern United States it does not properly belong to this map because it is dependent upon soil rather than climate (p. 600).

3a. Tropical steppe and savanna.

3b. Temperate savanna.

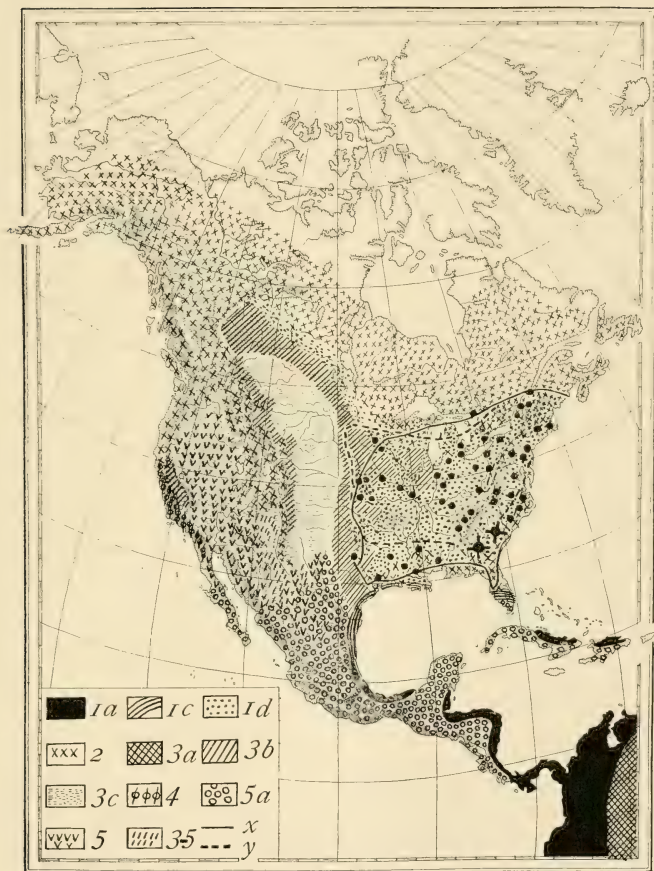
3c. Temperate grassland or steppe.

4. Evergreen forest with broad thick leaves.

5a. Scrub or thorny forest which merges into desert.

5b. Desert; 3-5 is very arid desert-like steppe.

Unshaded area in the north is tundra.



It is not to be understood that these forms are in any way directly related to the trees, but the trees represent the general conditions in which the beetles will live and reproduce. The species is an inhabitant of one of the 'climatic' realms and will be found continuously distributed where the forests are continuous.

6. *Geographic variation in life-history.* *C. sexguttata* rarely appears in northern localities in the autumn and it is probable that it remains in the pupal burrows until spring. The species is reported as appearing both autumn and spring in some southern localities. At Chicago, the adults appear during April and May, while in the western part of the geographic range of the species they do not appear until late in June, after the heavy rains which soften the soil, so that the imagoes can dig to the surface.

#### d. *Other species*

1. *Experimental studies of habitat selection.* By similar methods, I have determined the breeding place of the following species: *C. scutellaris*, high, dry sand with a little humus, or sand which is not shifting; *C. formosa generosa*, slightly shifting sand; *C. lepida*, shifting white sand; *C. duodecimguttata*, very moist dark soil; *C. punctulata*, soils with some humus and moist at egg-laying time; *C. purpurea*, same as *punctulata*, but in moister places, not repelled by considerable grassy vegetation, bare spots necessary as breeding places. In every case the range of the adults is far wider than the breeding grounds.

2. *Geographic variation in habits.* In captivity the larvae of all the species studied at Chicago close the burrows near the mouth and go to the bottom when the soil is dry. Here they remain inactive until water is applied. No such closures have been noted in the field, except *C. lepida*, which lives on the dry sand dunes. Criddle ('10) says:

In Manitoba, there are often long intervals of inactivity of the larvae of *manitoba*, *venusta*, *limbata*, *lecontei*, and probably others, during the summer months. At such times the larvae close their burrows at

the top, and remain apparently without food, and do not grow appreciably. In 1907, larvae of *venusta* and *limbata* closed their holes on June 12, and some did not appear again until August 25, nearly two-and-a-half months. A few, however, would open up at night, throw out a lot of earth, and then retire again. These larvae were always active when dug out. This strange habit may be due to the dryness of the soil to some extent, though it is not altogether so, as holes have remained closed during wet weather, and they are always opened in autumn or late summer, and deepened before winter, no matter what the condition of the ground is. The extreme heat of the sun may also be a factor of some importance. The beetles are unquestionably influenced by temperature, and will go into winter quarters earlier on a dry, hot fall than they do during a cold one, and hot summer days are much preferred for commencing winter homes.

The following table shows the depth of burrows at Chicago and at Aweme, Manitoba. It includes all available data.

TABLE 9

*Showing the relative depths of hibernation burrows of adults and burrows of larvae, of the same species, in the same soil. Manitoba, Criddle ('07, '10). Compare with table 10*

SPECIES	KIND OF SOIL	LARVAE		KIND OF SOIL	ADULT HIBERNATION	
		CHICAGO	AWEME		CHICAGO	AWEME
		Depth of Burrow	Depth of Burrow		Depth of Adult in Hibernation	Depth of Adult in Hibernation
		cm.	cm.		cm.	cm.
<i>C. limbalis</i> .....	clay	5-10	15-20	clay	5-8	7-15
<i>C. tranquebariae</i>	sand?	22-50	43-50	clay	15	15-30
<i>C. formosa</i> .....	sand	30-50	125-200	sand		62-117
<i>C. scutellaris</i> ....						
<i>lecontei</i> .....	sand	25-45	70	sand		25-64
<i>C. lepida</i> .....	sand	60-90	145-175			
<i>C. 12-guttata</i> ...	alluvial					
		5-10	15-37	clay	10-15	5-25
<i>C. repanda</i> .....	sand	10		clay	5-10	15-20

TABLE 10

*Showing the comparative meteorological conditions in Chicago and Brandon (25 miles northwest from Aweme) during active period of tiger beetles, April to September, and during the time of digging hibernation burrows (September)*

	RAINFALL: APRIL- SEPTEMBER	TOTAL SUNSHINE APRIL-SEPTEMBER	MEAN MAX. TEMP. APRIL-SEPTEMBER	RATIO OF RAINFALL TO EVAPORATION	MEAN TEMPERA- TURE	MEAN MAXIMA: SEPTEMBER	MEAN MINIMA: SEPTEMBER	TOTAL RAINFALL
	in.	hrs.	deg.	per cent. about	deg.	deg.	deg.	in.
Chicago.....	19.3	1695	70	100	48	71	57	33.4
Brandon.....	12.45	1310	68	80	33	66.4	38	17.45

On comparison of the data for the two points in question in table 10, we see that the amount of rainfall, the extremes of temperature and the length of season as well as the amount of sunshine differs widely at Chicago and in the vicinity of Aweme, Manitoba. Comparing these data with those found in table 9, we note that the larval burrows are deeper in the climate which is most arid and coldest in winter. Likewise the depth of the hibernation burrows is greatest where the temperature is lowest during the period of digging.

The shorter season, fewer hours of sunshine, and drought accompanied by the periods of inactivity described by Criddle may be the cause of the longer life-histories referred to in the case of the three species especially considered here.

#### C. GENERAL CONSIDERATIONS

We have noted various features of tiger beetle behavior. A discussion of the general bearing of this will now be presented under the following heads: (1) Importance of the breeding instincts and breeding place, (2) the relation of behavior characters to habitat and associated forms, (3) the meaning of variation in behavior, (4) relation of local and geographic distribution; importance of various factors.

1. *The importance of the breeding instincts and the breeding place*

We have shown that the adults range over an area much greater than that which the larvae inhabit and that a species is entirely absent where feeding habitats of the adults is represented and the egg-laying place or larval habitat absent.

Those tiger beetles which hibernate in situations different from the one in which the larvae are found, always return to the breeding place to deposit eggs. When the breeding place disappears, the species also disappears. The larval habitat or egg-laying place is much narrower and more definitely circumscribed than any other part of the habitat. The *breeding place* and the *breeding instincts* are, then, *the center about which all other activities of the organism rotate*. They are the axis of the environmental relations of these organisms.

a. *Comparison with other activities and relations.* The breeding place and breeding instincts must usually be considered in connection with the feeding ground, and feeding instincts as well as other factors. The tiger beetles will not breed where there is not sufficient nourishment for considerable periods. The feeding place is often the second consideration after breeding. In the tiger beetles, however, the feeding structures and habits are so generalized that their food is plentiful everywhere, and the food relations need only be mentioned. A third important environmental relation is that to means and place of escape from those environmental factors which tend to destroy the organism, such as its enemies, extremes of weather or climate, etc., but all these are of secondary importance.

b. *Fixity of breeding instincts.* The determination of their degree of modifiability or fixity would require experimental work which I have as yet been unable to accomplish. There is, however, good evidence from field study that the breeding instincts are most fixed of all the instincts. Since such behavior characters in the tiger beetles are usually specific or racial, they are probably modified only by the gradual processes of evolution.

## 2. *Relation of the behavior to habitat and associated forms*

There is the greatest difference in the behavior of the different species. I have as yet been unable to study this critically, but it is at least a very promising field.

*a. Behavior and habitat.* As we have noted, *C. sexguttata* has for example, various peculiarities of behavior which are related to the forest conditions in which it lives, which are not possessed by other forms. As we noted, when it is frightened from a rock or bare place, it frequently alights on the leaves of a low tree or bush and crawls under the bark of trees for the night, or even to hide when pursued. None of the other species which I have studied behave in such a manner. I have never seen *C. tranquebarica* crawl under objects when pursued. It does not alight on the green leaves of trees or shrubs when they are present. It excavates burrows instead of crawling under objects. The behavior of these two species is correlated with the general environmental conditions.

*b. Inter-physiology, and inter-psychology.* Tarde ('03) has recently written an article on inter-psychology—the psychology of the relations of individuals of the same species (man). To this should be added the behavior between different species, while acting or living together as one. He suggests that the social psychology of man may be traced to the inter-psychology and physiology of the lower animals. If this is true, then we can be more certain that the inter-psychology of the higher forms has developed from the inter-physiology of the lower forms (Craig, '08 [2]).

I have looked for the inter-physiological manifestations in these beetles, but have found none striking except the mating instincts. There is little or no social life. I have found animals belonging to totally unrelated species attempting to copulate in some cases where the two are dissimilar.

It seems quite evident from my observations that the more marked phases of the behavior of the tiger beetles arise not from inter-physiology, but from relations to the species which are quite

different in behavior and habit from the beetles themselves. This I propose to call *intermores-physiology* or psychology.<sup>5</sup>

c. *Intermores-physiology*. We have seen the behavior of these beetles when pursued, their flight, alighting only to wait for the moving object to come near when they start up again, the hiding under leaves of *C. sexguttata*, etc. All this is illustrative of the behavior which is related to forms antagonistic in behavior and habits.

The study of the behavior of forms which live together in the same situation from the point of view of the relations of the behavior of the different species is a promising field of investigation. It will throw much light on the problems of psychology as well as ecology.

### 3. *The meaning of variation in habits*

We have noted geographic difference in the length of the life-history and the depth of the burrows. In 1908 we pointed out that severe conditions increase the length of the various stages. Criddle has noted that the larvae do not feed for a considerable period in the summer. This accords fully with my experimental results on the larvae of the Chicago species. They stop feeding and close their burrows when the soil becomes too dry, or the condition otherwise severe. The lengthened life-history of Manitoba forms may be due to the shorter seasons and the failure of the larvae to feed for a considerable period.

We pointed out also ('08) that the larvae respond to stimuli by deepening their burrows. The soil conditions in Manitoba have not been studied, but the different depths of the burrow under different experimental conditions is suggestive.

The correspondence between experimental results and the differences in the so-called habits in the different localities suggests that the apparent *variation in habits* may be only a regulatory *behavior response* that probably would be found common to most individuals of the species. This could be settled by experimental study.

<sup>5</sup> *Mores* (Latin), 'behavior,' 'customs,' 'habits.'

#### 4. *The relation of local and geographic distribution*

We have suggested in the case of the three species here considered, that the geographic distribution of each is the geographic distribution of the conditions in which it lives and breeds. We have visited several of the different climatic realms in which each of these three and many other species occur. So far as ordinary observation can go, the breeding and general living conditions are similar in the different localities, even though the climate, as in the case of *Cicindela tranquebarica*, is very different. The same conditions are found by the species through its moving near to water in the arid climates, as compared with the more moist climate.

It is customary to conclude that *conditions* are the same because the *species* is the same. Here we have tried in a general way to determine whether the species is the same throughout its range, by the study of the condition, and experimentation on the animals. This is the only mode of attack that can yield definite results. It is highly desirable, however, to carry the observations on soil and other environmental factors further with the use of physical factor instruments. It is equally desirable to conduct actual experiments on each species of beetle at a number of points, especially those near the outskirts of its geographic range. This would, no doubt, reveal differences of detail which we have overlooked, but which cannot, so far as present observation goes, be of great importance.

#### 5. *Factors governing geographic distribution*

Our data show clearly that the breeding period is crucial as determining the local distribution, and that an excess or deficiency in any one factor is sufficient to decrease the number of individuals present, or cause them to be absent entirely. Any factor differing sufficiently from the optimum for a given species is sufficient to limit its distribution. There can be no important difference whether a deficiency in moisture is due to insufficient rainfall or to distance from or height above water, or whether an excess of temperature is due to latitude or exposure and accordingly the *same factors must govern both local and geographic distribution* of the tiger beetles.

### III. THE PHYSIOLOGICAL CHARACTERS AND DISTRIBUTION OF GROUPS OF SPECIES (FORMATIONS)

#### A. ZOOLOGICAL OPINIONS AND DIFFICULTIES

There is, I believe, a general opinion among laboratory zoologists to the effect that no important generalizations can be made from data concerning the environmental relations of animals. In other words, the data of natural history cannot be organized into a science.

There are at least three good reasons for the prevalence of such views. The first of these is that such zoologists are often familiar with only a few of the very common species of animals, common because their habitat relations are such that they can flourish in the conditions which civilization produces or because they do not have *definite* habitat relations, being in this respect an exception to the rule. The lack of attention to the taxonomy of common forms is also a factor. Animals which belong to different species, genera, or even families, are often quite similar in appearance and so are sometimes regarded as single species. Articles regarding American species have occasionally been published under the names of European species not found in this country, or at least rare and confined to northern latitudes.

The second reason results from the fact that relations of animals to their environment are not understood. Often we do not discriminate between the important and unimportant periods of relation to environment in a life-history. The third reason lies in the fact that the environment of animals is also not understood and the various stages and phases have not been classified so that habitat relations can be readily described.

The lack of knowledge of taxonomy and the simpler facts of natural history requires no discussion. On the other hand, our knowledge of animal behavior and animal physiology has been but little applied in the study of animals in nature, and the knowledge of environments, which is in the hands of the plant ecologists and geographers is not at all well known among zoologists.

## B. THE NATURE OF THE ENVIRONMENT

The animal environment is a complex of many factors. Each is dependent upon another or several others, in such a way that any change in one factor affects several. Some of the most important environmental factors are water (atmospheric moisture), oxygen, carbon-dioxide, nitrogen, temperature, pressure, currents, excretory products, food, enemies, and materials for abode (soil, vegetation, etc.). In nature, such combinations of these and other factors, in the proportion requisite for the maintenance of the life of a considerable number of animal species, are called environmental complexes (Davenport '03).

## C. ENVIRONMENTAL RELATIONS OF ANIMALS

The only features which space will permit us to discuss are the physiological and ecological relations. In this field we must confine ourselves to a comparison of plants and animals and the bearing of the important environmental relations on geographic distribution.

1. *Comparison of the environmental phenomena of plants and animals*

An organism is a system of inter-dependent and definitely related processes (i.e., a system in dynamic equilibrium). The definite relations of the inter-dependent processes of the organism (dynamic equilibrium) may be disturbed by changes in the external forces which surround the organism and to which the processes are adjusted (Jennings, '06). Such a disturbance is what we ordinarily call stimulation.

With this idea as a background, we give in parallel columns a comparison of the more obvious relations of plants and animals to their environments, as shown by experimental work. The column on the right is written by Dr. H. C. Cowles, Associate Professor of Plant Ecology in the University of Chicago.

TABLE II

*a Comparison of the responses of (motile) animals and (sessile) plants*

ANIMALS (MOTILE)	PLANTS (SESSILE)
I. Animal behavior is evident because of motility.	I. Plant behavior is inevident because of lack of motility.
II. When an external stimulus is applied to an animal, it responds mainly by movements which are usually more or less random, and which bring the organism into various conditions, one of which relieves the disturbance and the organism resumes normal activity, in conditions which brought the relief. These conditions are not necessarily advantageous. . . . (Jennings.)	II. Plants respond more evidently through changes in form and structure.
III. Animal behavior is usually plastic, i.e., may be modified by external stimuli, but sometimes appears rigid.	III. Plant structures are usually plastic but frequently appear rigid.
IV. (Animal structure is only slightly plastic. The plasticity usually occurs in the early stages). Structural modifications are rarely of importance in the life of the animal.	IV. Structural modification of plants is often of importance in the life of the plant.
V. The motile organisms of a given habitat usually have common behavior characters. Combined structural and behavior characters of comparable forms of a given habitat, or of similar habitats are ecologically equivalent. <sup>5</sup>	V. The plants of a given habitat usually have common structure and functions, or those that are ecologically equivalent. <sup>6</sup>
VI. The breeding activities of animals are probably least modifiable and least regulatory, but are governed by the same laws as the other activities. (Shelford, '07, '10).	VI. The reproductive organs and embryonic stages of plants are less modifiable than the vegetative stages of adults.

<sup>5</sup> The meeting of the same general conditions in a different way constitutes ecological equivalence. The term was first used by Cowles.

b. *Discussion of the parallel statements.* (II) Animal (or motile organism) distribution at any given time is a better index of the condition at that time than the distribution of plants, because when the conditions at a given point become unfavorable, the animals (or motile organism) move to another situation, while the plants (or sessile organisms) remain or die.

(V) The fifth is not well established. However, a preliminary testing, for example, of the rheotaxis of a large number of brook animals has shown them to be strongly positive, strong positive rheotaxis being a common behavior character. Many of them have special means of attachment which may be brought into play with great speed.

The darters are strong swimmers and are able to live in rapids by virtue of their swimming powers and positive reaction, while the snails (*Goniobasis*) which occupy similar situations, are able to maintain themselves because of the strength of the foot and positive reaction. The two are ecologically equivalent. The sixth statement appears to be generally true, but needs experimental confirmation.

The proposition may be summarized as follows: The behavior and general mode of life of animals are the superficial equivalent of the structural phenomena in the vegetative parts of plants. Behavior and vegetative structure are convenient indices of physiological conditions within the organism.

To illustrate this still further, let us consider the plants of the sand areas at Chicago and in Manitoba. As compared with Chicago plants, the plants of Manitoba differ in size and structure under the more arid conditions found at the point where Criddle's studies were made. The Manitoba tiger beetles do not, so far as I can find, differ from the Chicago forms in any of the structural characters which have to do *with their meeting those conditions, but they dig their holes deeper and require longer time for transformation.* The tiger beetles of the desert and semi-desert and the tropical sand areas (Bates and Westwood, '52; Snow '77; Lucas, '83) are usually nocturnal or crepuscular; those of moister and cooler areas are diurnal—a difference in behavior. Desert plants are structurally adapted to withstand the desert conditions

(Schimper '03) and differ in this respect from plants of cooler, moister situations. Again, the difference between the tiger beetles which deposit their eggs in different soils is not structural difference in ovipositor, but a different physiological response of the organism.

The activities mentioned are general and may be separated, into feeding, breeding, etc. Probably all are governed by the same general laws. In the study of all the animals of a given environment we are confronted with the question of what activities are most important, just as in the study of particular species.

## *2. The most important environmental relations of animals*

The strength of a chain is the strength of the weakest link. The activity which determines the range of conditions under which a species will be successful is the activity which takes place within narrowest limits. Failure to recognize this principle is in part responsible for the general chaotic state of our knowledge of natural history. Men have often failed to interpret the relations of animals to their environments because they have regarded the records of the occurrence of all stages of the life-history as equally important. They have considered the occurrence of the most motile stage in the life-history important, as for example, the occurrence of an adult butterfly. Plant ecologists would have met with equal success if they had studied only the environmental relations and distribution of wind-disseminated seeds. While we have indicated above that the breeding activities are most limited (Merriam, '90; Allen and Verrill fide Merriam, '90; Adams, '08; Shelford, '07, '10; Reighard, '10; Herrick, '02; Clark, '10), there are no doubt exceptions to this, and at the present stage of our knowledge the subject is one for investigation. Whatever this activity may prove to be in a given case, it is of great importance and deserves comment, both as to method of investigation and bearing on distribution.

*a. Method of determining the most important activities.* The first step is field observation—the continuous watching of animals throughout a number of life cycles. Experimentation is almost

always necessary also. It is only under unusually favorable conditions that the relative importance of the various periods of the life-history of an animal can be ascertained, without experimentation. On the other hand, experimentation must be correlated with field observation. Simple experimentation on the behavior of animals in the laboratory does not illuminate this matter to any appreciable extent.

### 3. *The relation of physiological characters to geographic range*

Our studies of animal distribution usually consist of a list of names of species with a statement of the distribution of each, followed by such interpretation as suits our particular purposes. Attempts actually to study the environment in any detail, or the reactions of animals to the conditions of environment are rare indeed. Furthermore, the groups most studied (higher vertebrates) are probably least dependent upon their environmental complexes; they are often decidedly migratory and because of their size least adapted to experimental study.

Some quite extensive attempts to correlate geographic range with meteorological conditions have been made, but always with only implied reference to the physiological character of the organisms themselves, and usually with the use of *species* as an *index of conditions*. A few factors have been emphasized, and these usually in the sense of barriers. Merriam ('90, naming also Allen and Verrill but not citing their papers) emphasizes temperature; Walker ('03) atmospheric moisture. Heilprin ('87, p. 39), like most paleontologists, emphasizes food. There appears to be no adequate basis for the idea that the same single factor governs the distribution of most animals. Such a conclusion probably results from leaving the organism out of consideration.

Since the environment is a complex of many factors, every animal lives surrounded by and responds to a complex of factors, at least in its normal life activities within its normal complex (Jennings, '06, p. 180). Can a single factor control distribution?

A large amount of physiological study of organisms has been conducted with particular reference to the analysis of the organism itself, but with little reference to natural environments.

Many of the factors and conditions employed in such experiments are of such a nature that the animal never or rarely encounters them in its regular normal life. Other experiments are, however, attempts to keep the environment normal, except for one factor (Jennings, '06, p. 180). These have demonstrated that in ordinary reactions an animal responds to the action of a single stimulus. Certain general laws govern the reaction of animals to different intensities of the same stimulus.

*a. Laws governing the reactions of animals.* The laws governing the stimulation of animals in the experiments of the laboratory are familiar subjects in the textbooks of physiology (Verworn-Lee '99). With respect to a given factor used in the experiment, it has been found that there is a range of conditions within which the activities of the animal proceed without marked stimulative features. These are called optimal conditions. Take, for example, temperature. There is in most animals which have been subjected to experimentation with temperature, a range of several degrees in which the animal is not markedly stimulated (optimum). As the temperature is raised or lowered from such a condition, the animal is stimulated. If the temperature be continuously raised, a point is reached at which the animal dies. The temperature condition just before death occurs is called the maximum. The lowering of temperature produces results comparable in a general way to those of high temperature. The condition just before the death point is reached is called the minimum. With various limitations, unimportant in this connection, the same is true with respect to each of the various factors which an animal encounters in nature. Which factor determines the limitations of occurrence of an animal on the earth's surface? The answer to this is suggested in Liebig's Law of Minimum.

*b. Law of minimum.* Liebig's law of minimum is summarized by Johnstone ('09, p. 234):

A plant requires a certain number of food stuffs if it is to continue to live and grow. Each of these food substances must be present in a certain proportion. If it is absent the plant will die; if present in a minimal proportion the growth will also be minimal. This is true no matter how abundant the other food stuffs may be. The growth is then dependent upon the amount of food stuff present in minimal quantity.

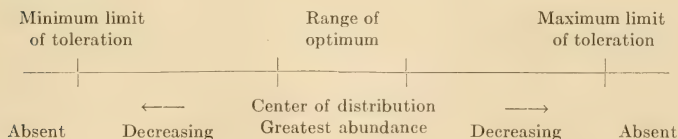
In nature this law applies both geographically and locally. As applied to animals it includes both food and material for abode. The presence, absence and success of a species is determined by the necessary material which is absent or present in minimal quantity.

c. *Law of toleration of physical factors.* We have noted (p. 581) in the case of the tiger beetles, that for the egg-laying to take place the surrounding temperature and light must both be suitable, the soil must be moist, probably also warm, and must satisfy the ovipositor tests with respect to several factors. Egg-laying, the *positive reaction*, is then probably a response to several factors. Furthermore, after the eggs are laid, the conditions favorable for egg-laying must continue for about two weeks if the eggs are to hatch and the larvae reach the surface of the ground. The success of reproduction depends, then, upon the qualitative and quantitative *completeness* of the complex of conditions. The *negative reaction*, on the other hand, appears to be different. The absence of eggs, the number of failures to lay and therefore the number of eggs laid in any situation can be controlled by qualitative or quantitative deficiency or excess with respect to *any one of several factors*. The presence, absence, or number of eggs laid is, then, determinable by a single factor, according as it is near the optimum or near either the maximum or minimum tolerated by the species. It is, however, not necessary that a single factor deviate; the effect is similar or more pronounced if several deviate.

In nature the presence or absence, or success of a species or group of species, its numbers and sometimes its size, etc., are largely determined by the degree of deviation of a factor or factors from the range of optimum of the species or group of species. The cause of the deviation in the factor or factors is not of importance. For example, in the case of a soil inhabiting species such as *Cicindela tranquebarica*, to which considerable moisture is necessary, the cause of the deficiency in one case may be climatic deficiency in rainfall, in another a rapid drainage due to steep slope and porosity of soil. The former is what we have called a climatic (geographic) condition and the latter a

local condition. The evidence for the law of toleration as applying to distribution is good so far as the local distribution is concerned and, since the same factors are involved in the geographic, there is no difficulty in the application of the law to geographic distribution also. The fact that in so far as our observation can go at present, the tiger beetles are found in similar conditions throughout their ranges, is also good evidence for the application of both the laws of minimum and toleration to geographic distribution. In fact the *law of minimum* is but a special case of the *law of toleration*. Combinations of the factors which fall under the law of minimum may be made, which makes the law of toleration apply quite generally; for example: food and excretory products may be taken together as constituting a single factor. From this point of view the law of toleration applies, the food acting on the minimum side, excretory products on the maximum.

d. *Application of the law of toleration to geographic distribution.* The so-called centers of distribution are often only areas in which conditions are optimum for a considerable number of species (Transeau, '05; Adams, '02 and '05). The relation of the law to centers of distribution is shown in the diagram below; above the line is the scale of stimulation with the limits of toleration shown and below the parallel relation of the distribution and relative abundance.



On account of the nature and distribution of climatic and vegetation conditions, it follows that as we pass in one direction from a center, one factor may fluctuate beyond the range of toleration of a species under consideration; but as we pass in another direction it is very likely to be a *different* factor. The divisions of Merriam's zones into arid and humid portions is an illustration of this, and seems to constitute a begging of the temperature question.

#### 4. *Tentative laws of distribution*

On this general basis tentative laws of distribution may be formulated.

a. *Governing the limit of geographic range.* The geographic range of any species is limited by the fluctuation of a single factor (or factors) beyond the limit tolerated by that species. In non-migratory species the limitations are with reference to the activity which takes place within the narrowest limits. In migratory species this activity limits the range only during a part of the life cycle.

b. *Governing distribution area.* The distribution area of a species is the distribution of the complete environmental complex within which it can live as determined (1) by the activity which takes place within the narrowest limits and (2) by the animal's power of migration. Barriers in which some one factor of the complex fluctuates beyond the limits of toleration of the species at all periods of its life-history may prevent the animal from reaching all the suitable habitats, but this is the result of the working of the laws rather than an exception, and faunistic animal geography begins where physiological animal geography ends.

#### D. CLASSIFICATION OF ANIMAL ENVIRONMENTS

While this is a necessary subject for discussion, it is with much hesitancy that I undertake it here, where brevity is necessary. Obviously, since our subject is physiological animal geography, we shall confine our attention mainly to those aspects which are geographic in extent in the sense that they are nearly uniform over a considerable area of the earth's surface.

If one is to understand the most elementary principle of synecology,<sup>7</sup> he must first recognize the distinction between *local* (edaphic, Schimper, '03; minor and secondary, Adams, '08), and *climatic or geographic (extensive) environmental complexes (major,*

<sup>7</sup> Synecology is the ecology of formations. In the classification of formations and environments, no nomenclature has been established for the larger or climatic units. Dr. Cowles tells me that plant formations do not represent climate and therefore 'climatic' should not be used. However, every ecologist and geographer knows the significance of 'climatic' and 'local.' The geographers object

Adams, '08). The climate of a region and all that goes with the climate are a climatic or geographic complex. Opposed to these are local complexes, such as water (streams or lakes), soil, exposure, or lack of exposure, etc. For example, in the Mohave Desert the climatic conditions are characterizable as hot, arid, etc., but within the desert are streams fed by mountain rainfall. These streams are local conditions in themselves, and also produce other local conditions such as moist soil, etc. These are not dependent upon the dominant conditions within the desert.

The relation of local and geographic condition has been the subject of much careful consideration by Cowles ('01), Schimper, ('03), Shelford ('07), Adams ('08) and ('09).

We will turn our attention, then, first to an inquiry as to the best index of climatic or geographic condition or major environmental complexes.

*1. The index of climatic or geographic conditions or of major environmental complexes*

The vegetation from the standpoint of whether it is forest steppe, or desert, etc., does not involve animals, and represents climatic complexes in a general way. It is the most important factor in the control of temperature, moisture, light, food and material for abode<sup>8</sup> and is a good index of the conditions which surround animals. Tentatively it may be used as a basis of classification of the animal environments. A knowledge of these environmental complexes may be acquired from the data of physiography, meteorology, plant ecology and physiological plant geography (Schimper, '03).

to the use of the term 'geographic' for the climatic environments because, to them, the local environments are equally geographic. Every zoologist understands the meaning of 'geographic' and 'local.' Adams' terms, 'major' (climatic) and 'minor' (local) are to be preferred but one must continually explain their meaning. The writer uses 'climatic' and 'geographic' here because their meaning is clear.

<sup>8</sup> Material surroundings have been regarded as of great importance in the case of mammals. Hagenbeck states that he always supplies an environment which resembles as far as possible the natural environment. He has imitation icebergs for polar bears, etc., and finds that this adds greatly to the success of keeping his animals in captivity.

## E. THE ANIMAL FORMATION

Animals select their habitats, probably by trial and error, as is indicated by the making of additional holes and parts of holes by the tiger beetles only to reject them without laying eggs. The simple fact of selection is, we believe, very familiar to all naturalists.

A given environmental complex is selected by a number of species. All of the animals of a given habitat constitute what is known as an animal formation (Warming, ('09); Clements, ('05); Schimper, ('03); Adams, ('08); Grisebach, ('48, *fide* Clements).

It follows that there is a certain physiological or ecological similarity and ecological equivalence in the forms that thus select the same or similar complexes. It follows<sup>9</sup> that the animals of different deserts, different deciduous forests, different steppes, etc., are ecologically and physiologically similar or ecologically equivalent if the deserts, the forests, and the steppes, etc., are similar (Adams '05).

Tentatively, formations may be characterized in general physiological or ecological terms (*mores*). The characterizations of plant formations have thus far been largely based on *growth-form*. Attempts to find structural similarity among animals of similar habitats, while not failing in particular cases, have led to no good results or generalizations (Ritter, '09). The great difficulty with this point of view is that it must, because of the great difficulty of investigation, remain for a long time largely a matter of speculation. The attempts which have been made are based on natural selection speculations or Lamareckian speculations.

It should be noted further that the relations of a given group of animals to their habitat and to each other is more complex than that of the plants which are commonly treated in this manner.

The conspicuous plants of a given environmental complex, except in the tropical forests, are usually rooted in a single plane which greatly simplifies the relations of plants to their environments.

<sup>9</sup> A term is needed to cover such characters. The term *mores* (Latin), 'customs,' 'behavior,' 'habits' is suggested as best covering the need. It stands opposed to form and forms; thus steppe *mores* meaning the *behavior* of *characteristic steppe* animals or an animal or animals with characteristic steppe behavior.

Animals, on the other hand, have different habitats which are not related to one plane, and so must be separated into similar groups for purposes of the comparison of one formation with another. For example, the animals which burrow into the ground in a given environmental complex cannot be compared with those that live in trees in another, but must be compared with subterranean forms. Accordingly, for comparison, animals must be separated into: (a) burrowing forms, (b) ground forms, (c) arboreal forms, etc.

### 1. *Classification of animal formations based on environmental relations*

*a. Principles of classification.* We have noted that all of the animals of a given environmental unit constitute a formation, and that environmental units are classified into climatic or geographic (extensive) and local. The groups of animals which occupy the climatic or geographic environments may be called 'climatic or geographic animal formations.' The groups of animals which occupy the local environments are called local formations (societies, or associations).

If one is to study the relation of animal physiology and behavior to the environmental conditions, in so far as this can be done by field study, these distinctions must be kept clearly in mind. For example, in dealing with animals of the great North American steppe area, to treat together all forms found here (as is common practice) would lead to endless confusion from our point of view. The forms which belong to the water (aquatic), those that live in the timber along the ravines, in the sand areas, are forms belonging to *local formations*. Those that occupy the plains proper belong to the *steppe formation*. Some forms may belong to both, in which case the facts should be taken into account.<sup>10</sup>

<sup>10</sup> An animal should be associated: first, with the breeding conditions; second, with the feeding conditions; third, with the conditions affording shelter. Calvert ('08) attempted to find correlation between the distribution of Odonata and vegetation zones with negative results. Aside from the reasons given by the author, it should be noted that Odonata breed in the water and, excepting forms breeding in water holding plants, belong to local conditions, and no correlation was to have been expected. Correlation of the distribution and species is, however, not essential to our point of view.

b. *Climatic or geographic animal formations of the world based upon physiological similarity and ecological equivalence under similar conditions.*<sup>11</sup> The distribution of the similar environments is given by Schimper ('03) and Transeau ('03, '05) and in fig. 19. Only the environments and distribution of the formations is given here; much concerning the *mores* of the different formations may be obtained from the existing literature but we do not have it well enough organized to present here.

- 1 Formations of forests with broad, thin leaves.
  - a Tropical rain-forest formations (fig. 19, 1a).  
 Environment: Dense forest with broad thin leaves, two or three heights of trees, uniformly distributed rainfall and nearly uniform temperature.  
 Distribution: Large areas Mexico and Central America (Belt, '88),<sup>12</sup> and South America (Bates, and [Clodd, '93]), southern Asia and East Indies (Wallace, '94), and several small areas in Africa (Garner, '01).
  - b Monsoon-forest formations.  
 Environment: Similar to the rain-forest but with a dry season in which the leaves fall.  
 Distribution: Adjoins areas of rain-forest.
  - c Temperate rain-forest formations (fig. 19, 1c).  
 Environment: Similar to the tropical rain-forest, but much less luxuriant and in different climatic conditions.  
 Distribution: East coast of northern Mexico, southern U. S., western Chile, southern Japan (Kobelt, '02), New Zealand.
  - d Temperate deciduous forest formations (fig. 19, 1d).  
 Environment: Similar to the temperate rain-forest, but much less dense and deciduous.  
 Distribution: Eastern North America, north to the Great Lakes; Chile, north to 35° (Darwin, '45, p. 242); Europe, north of the Alps (Mosley and Brown, '63, p.) and south of 60° (Kobelt, '02; Brehm, '06); Japan and vicinity of Okhotsk.
- 2 Formations of forests with narrow, thick leaves (coniferous forest formations; further study will probably subdivide these) (fig. 19, 2).  
 Environment: dense evergreen forests with little undergrowth.  
 Distribution: North America, north of the Great Lakes and Columbia River extending southward in the mountains (Seton, '09); Eurasia, north of 60° and southward in the high mountains (Brehm, '96).

<sup>11</sup> This outline is essentially that arranged for a committee of the Geographic Society of Chicago on the Classification of Geographic Materials, and is parallel to one for plants by Dr. H. C. Cowles and to one for Human Geography by Dr. J. P. Goode and Miss J. B. Obenchain.

<sup>12</sup> Some characteristic literature on the natural history of the various formations is cited where possible. See Thomson's introduction to Brehm ('96).

- 3 Formations of savannas and grasslands.
  - a Warm savanna and steppe formations (fig. 19, 3a).  
 Environment: Dry season in spring; scant rainfall; grassland with scattered thorny trees, occasionally thickets, and dense forests along larger streams.  
 Distribution: The great plains of Africa (Roosevelt, '09-'10), and South America.
  - b Cool savanna formations (fig. 19, 3b).  
 Environment: Similar to the warm in aspect, but more often with trees in groves.  
 Distribution: A narrow belt nearly surrounding the Great Plains, Uruguay, Australia, and eastern Siberia (Brehm, '96).
  - c Cool steppe formations (fig. 19, 3c).  
 Environment: Cool, dry, winters cold, grassland with trees only along the principal streams.  
 Distribution: The great plains of North America (Craig, '08; Seton, '09), south central Asia (Brehm, '96), De La Plata southward to Patagonia (Hudson, '92).
- 4 Formations of forests with broad, thick leaves (fig. 19, 4).  
 Environment: Subtropical conditions with winter rain and hot, dry summers.  
 Distribution: California, the Mediterranean region, Chile (near Valparaiso, Darwin '45), South Africa, southwest Australia.
- 5 Formations of deserts and scrub areas (semi-desert).  
 Environment: Various types of arid condition with thorny vegetation.
  - a Scrub or semi-desert formations (fig. 19, 5a).  
 Distribution: Mexico, Texas and Central America (Belt, '88; Bailey, '05), eastern Brazil, southern South America, arid Australia (in part), northeastern Africa (Plowden, '68), India, and China.
  - b Desert formations.  
 Distribution: Southwestern North America (Merriam, '90), South America, Sahara and Arabia (Brehm, '96), central Australia and south Africa.
- 6 Tundra formations.
  - a Arctic tundra formations.  
 Environment: Cold, treeless, with short cold summers.  
 Distribution: Circumpolar (fig. 19).
  - b Alpine tundra formations.  
 Environment: Similar to *a*.  
 Distribution: Mountains above the tree line.
- 7 Formations of fresh water.
  - a Still water formations (lakes, ponds and sluggish streams).
  - b Turbulent water formations (swift streams and eroding lake shores).
- 8 Formations of the sea and its shores (amphibious formations, principally breeding on shore, feeding in sea).
  - a Ice-bound shore formations (Arctic) (Brehm, '96; Shackleton, '10).
  - b Tropical and temperate shore formations.
  - c Oceanic Islands formations (the island fauna, representing the migration of land animals by sea) (Wallace, '92).

9 Formations of the waters.<sup>13</sup>

- a Formations of the sea (marine) (M'Intosh, '04).
  - 1 Limestone bank formations (littoral) (Brooks, '93).
  - 2 Rocky (eroding) shore formations (Littoral) (Verrill, '72; King and Russell, '09).
  - 3 Sandy (depositing) shore formations (littoral).
  - 4 Open sea formations (pelagic) (Heilprin, '81).
  - 5 Deep sea formation (mudline and abyssal).

It should be noted that the various formations of the list are to be found duplicated or essentially so in various parts of the world. This point of view emphasizes the *resemblances in the behavior and ecology of forms living under similar conditions*. In the case of the great zoogeographic regions, there is *no duplication*, and *differences* are emphasized.<sup>14</sup>

<sup>13</sup> The distribution of aquatic animals is governed by: a. Kind of bottom (Sumner, '09). b. Depth, current, temperature and all other factors which are modified by depth, etc.

<sup>14</sup> There are no doubt several valid objections to such a classification, when thus statically stated and as mapped by some workers, such as Schimper. We present it thus because the *recognition of the existence and general features of a phenomenon must precede its analysis*. However, one of the most important of these objections arises when one inspects a number of maps of the distribution of species. Such an inspection shows that the distribution areas of some species are bounded by the limits of the deserts, steppes, forests, etc., while those of others bear no relation to these regions. The former afford no difficulties while the latter deserve further comment. Species that, apparently, do not fit our classification fall under three heads: 1. Species whose range is far greater than that of any realm or plant formation, covering perhaps several realms. 2. Species that occupy only a part of the plant formation in which they belong. 3. Species whose range lies within a region intermediate between two realms or plant formations.

The first group is made up of species *dependent wholly or in part upon local conditions*. Some species are always associated with local conditions, e.g., *C. tranquebarica*, p. 574, fig. 14. Such forms are relatively independent of climate, geographic plant formations, etc., and are dependent upon such conditions as are afforded by streams, sand areas, lakes, etc.

The species which are in part dependent upon local conditions usually belong properly to the climatic or geographic conditions of one formation, and invade another formation in local conditions which happen to be like the geographic of the one, in respects essential to that species. For example, some of the species of Orthoptera belonging to the great plains, or North American steppe region, invade the sand areas in northern Indiana where the climate is suitable for forests. Such phenomena are common and have been discussed by Adams ('02, '09).

## IV. THE PROBLEMS, METHODS, AND RELATIONS OF PHYSIOLOGICAL ANIMAL GEOGRAPHY

## A. SOME PROBLEMS OF PHYSIOLOGICAL-ANIMAL-GEOGRAPHY

1. *Behavior problems.* That the behavior of animals reflects their general conditions of existence, I think will not be seriously doubted. Some of the geographic problems may be stated as follows:

a. Behavior and geographic conditions. How much, and what features of the geographic conditions, for example, such as the steppe, the tundra, or the tropical forest, are reflected by the behavior of animals? Are these characteristics acquired by the individual or are they hereditary? In connection with the first question, I quote Brehm on the Arctic fox:

His whole character and conduct are quite different from those of our reynard and his near relatives. One scarcely does him injustice in describing him as a degenerate member of a distinguished family, unusually gifted, intelligent, and ingenious. Of the slyness and ingenuity, the calculating craft, of his congeners he evinces hardly any trace. His disposition is forward, his manner officious, his behavior, foolish. He may be a bold beggar, an impudent vagabond, but he is never a cunning thief or robber. He follows his worst enemy; without fear he approaches a man sleeping in the open, to snap at a naked limb.

The behavior of the penguins of Antarctica as described by Shackleton is equally interesting. Is it, or is it not, a picture of the hard struggle, intense cold, and monotony of the tundra?

<sup>14</sup>—Continued.

Our second group (or species which occupy only a part of the formation to which they belong) is important. Maps of the distribution of trees by Transeau ('05) illustrate this. An inspection of these shows that there is a central area in the formation, in which species are most numerous, and in which we may conclude the conditions for the majority of the forms are best (optimum). Suitable investigation would no doubt show that species thus narrowly distributed are limited by the termination of their necessary conditions, and that relative numbers are dependent upon the law of toleration.

Our third type, or species which occupy intermediate ground between the realms, are few so far as observation has been recorded (Ruthven, '07).

The above discussion is, however, based on the *distribution of morphological species*. If, however, there are *physiological differences*, *behavior differences*, or even *regulatory responses* in the different formations, morphological species and their distribution, are unimportant matters.

b. Inter-psychology and inter-physiology (between ecologically similar forms). The problems of the inter-psychology (Tarde, '03) and inter-physiology (p. 588) are equally important in connection with the relations suggested above. Some aspects of inter-psychology are not inter-specific, but concern forms with similar habits. In the steppes ecologically similar animals frequently act as one species. Mr. Roosevelt has said: "One of the most interesting features of African wild life is close association and companionship so often seen between totally different species of game" (Roosevelt, '09). Mr. Roosevelt shows the zebra and hartebeest herding together.

c. Intermores-psychology and physiology (between ecologically dissimilar forms, or antagonistic forms). The relations of animals of different size, habits, etc., to one another involves the most striking features of behavior. Much of the behavior which tends to protect the species from enemies falls under this head. This aspect of behavior has its geographic as well as its local significance. For example, the problem of the effect of the presence or absence of large carnivores on the behavior of other animals present in a climatic formation would deal with the broader geographic side.

d. Geographic variation of *mores*. The phenomenon of geographic variation in behavior and physiology probably usually belongs to wide ranging species. The best available data are probably on the nesting habits of birds (Knowlton '09).

2. *The more purely physiological problems.* Let us illustrate by the desert. The dominance of the reptiles in the desert is well known, and Dr. A. P. Mathews has called my attention to the fact that the excreta of reptiles is uric acid which is a substance of low osmotic pressure passing out with the feces in a dry state; little water is used in the disposal of the excreta. This, together with the thick skins, enables reptiles to meet the conditions of the desert. Desert mammals must meet the same conditions. In these, water is required to wash the urine out of the tubules. Mammals are few in the desert; their physiological relations there are not well known; Swain ('03) has pointed out the high specific

gravity of the urine of the California coyote. The fact that many mammals do not drink for long periods in the steppe and desert regions is well known. Livingstone ('58) noted it in the Kalahari Desert, Roosevelt ('09) in east Africa, and Craig ('08) in the case of the prairie dogs and birds of Dakota. (Verworn, '99 p. 280.)

## B. METHODS

The methods of physiological animal geography have been indicated from time to time throughout the paper. The method may be characterized as combined experimentation and field observation, each conducted with reference to the other, and both conducted with reference to *animal formations*. A typical study with reference to the steppe would consist of (*a*) a field study of a number of carefully chosen steppe species, accompanied by experimental study of their behavior and of their physiology; (*b*) a comparison with a similar study in a steppe in another part of the world; (*c*) a study of steppe species ranging outside the steppe with a view to ascertaining variation in behavior or behavior differences, etc.; (*d*) a comparative study of steppes and other formations.

## C. RELATION OF PHYSIOLOGICAL ANIMAL GEOGRAPHY TO OTHER SUBJECTS

The problems of physiological animal geography lie close to those of human geography, sociology and psychology, and offer a field of observation which may be accompanied by experimentation.

1. *Human geography.* Its relation to human geography is especially intimate. Indeed, geographers have been disappointed with the data which zoology has furnished them. It is almost exclusively data concerning the taxonomy and morphology of animals. The parallelism between the geographic phenomena in animals and the relation of culture to environment lies not in the color and structural adaptations of animals, but in their *behavior*

characters which enable them to live under a given set of conditions and the behavior which those conditions produce.<sup>15</sup>

It is to be hoped that geographic studies such as we have outlined may be conducted on wild animals in connection with the geographic and psychological problems in man (Waxwieler, '06).

2. *General biology and evolution.* From the biological side alone, the more purely physiological problems present an interesting field which is sure to yield results of far-reaching importance. The day is, I believe, rapidly approaching when the physiologist will find it necessary to give more attention to the study of animals unacclimated to the gases and artificial surroundings of the laboratory. Indeed, the failure of students of behavior to study their animals in nature is probably constantly leading to misinterpretation.

It has not been my purpose to point out the relations of this subject to the evolution of species. However, to the question of the evolution of behavior characters, of instincts, etc., this point

<sup>15</sup> While attempting to make comparisons between human society and man on the one hand, and plants and animals on the other, geographers, sociologists, and psychologists—in so far as I have been able to read their writings along this line—have compared structure in plants and animals with what is obviously not structure in man, namely, his culture and mental make up. Waxwieler compares human society with the whole animal kingdom as constituting another society. McGee ('96) takes a similar position. In discussing the relation of cultures to environment, he says: "When the law of biotic development is extended to mankind, it appears to fail; for the men of the desert and shore land, mountain and plain, arctic and tropic, are ceaselessly occupied in strife against environmental conditions which transform their subhuman associates, yet men remain essentially unchanged, some taller, some stouter, some swifter of foot, some longer of life than others, yet all essentially *Homo sapiens* in every characteristic.

More careful examination indicates that the failure of the law when extended to man is apparent only. The desert monads retain certain common physical characteristics, but develop arts of obtaining water and food, and these arts are adjusted to the local environment. . . . " He continues with the citation of other cases. In the light of our present knowledge, such adjustment of arts is comparable only to the adjustment of wide ranging species of animals in food, nest building, materials used in nest building, and other features of ecology and behavior (see also Hubbard '96; Mason '96). Goode ('04) called attention to the fact that physical changes in man are slow as compared with the changes in culture (see also W. S. Tower, '10).

of view is very important. While many aspects of these problems are not geographic, many others are, and the study of physiological animal geography bears the same important relations to the study of evolution of behavior as did faunistic animal geography to evolution of species in the beginning of its study.

The relations of animal behavior to the evolution of species has never been appreciated. It is obvious that the behavior of all animals is regulatory and tends on the whole to preserve the species and to retain it in the environmental complex to which it is adjusted; still only slight changes in the physiological characters of an animal will cause it to select a slightly different complex, open entirely new avenues of migration and change the distribution of the group of species to which it belongs. Such a change in physiological character would bring a group of species into an entirely different relation to all the so-called factors of evolution (McDougal, '08; Tower, '07, '10). Students of experimental evolution have, in no case that has come to my attention, made any study of the behavior characters of their new races, while the morphological features have been pursued with vigor. Is it not time that students of evolution began to study the effects of behavior on evolution?

#### D. THE FUTURE BIOLOGY

In this paper we have sharply separated evolution and structure on the one hand, from physiology and behavior on the other. Space, clearness, and the condition of the subjects have forbidden that we attempt to unite them here. While it may be expedient to continue in this manner until our knowledge of physiology and behavior is commensurate with that of the other subjects, the following of such a course indefinitely, with respect to either morphological or physiological aspects of biology cannot, if it be general, bring about the best development or unification of biological science. Indeed, its present lack of unity is traceable to such a course followed until recently by zoologists generally.

If our understanding of the data of physiological cytology be correct, we may expect to find so-called structures of some sort within or among the cells concerned in function, which stand for

or are correlated with each physiological state and physiological condition to which we have referred. Our methods may not at present be sufficiently delicate to detect such structure, or the processes which lie back of it, but we may, it is believed, confidently expect the necessary methods for the detection of such structures and processes, and especially their correlation with and relation to the more permanent and more easily recognizable morphological conditions.

We classify the responses and changes in animals as evolution, modification by the environment, behavior and physiological response. Are not all these, after all, but different expressions of the same or similar processes? Future investigations must answer this question and it is around this question that the future of much that is known as biology hinges.

## V. GENERAL CONCLUSIONS AND SUMMARY

### 1. *Distribution and dispersal*

a. Every animal selects an environmental complex as its general habitat (pp. 566, 571, 579).

b. The breeding grounds are usually the most important index of the true habitat (p. 587).

c. Each species is usually distributed as far as its environmental complex extends, unless barriers are encountered; faunistic animal geography begins where physiological animal geography leaves off (pp. 573, 582, 598).

d. The success of a species within a territory and its limitations to that territory are determined by fluctuation of one or more environmental factors, toward or beyond the limit tolerated by the species (p. 599).

e. Species which select those environmental complexes which are determined by streams, soil, or other situations which occur only locally, are local in their distribution (pp. 574, 5).

f. Animals which select a habitat which is *geographic in extent* and which represents the dominant conditions of an area, are distributed throughout their area and are usually not so wide ranging as the species which select the *local* conditions (p. 582).

*g.* The dominant vegetation of a given area which possesses some degree of uniformity of climate (as, for example, the deciduous forest of the United States), is the best index of general conditions, as it not only presents the results of the conditions, but makes certain types of environmental complex for the animals (pp. 582, 601).

*h.* The field of plant ecology and of ecological plant geography present the best data on the distribution of animal environmental complexes (p. 601).

## 2. *The physiology and behavior of animals*

*a.* In animals, behavior characters take the place of growth-form in plants. Animal formations may be characterized by the behavior, physiological, and habitudinal relations (*mores*) of the constituent animals, while plant formations are superficially characterized by structural characters which indicate the physiological conditions of the constituent plants (593).

*b.* Animal behavior, physiology and general mode of life (*mores*) probably reflect the geographic conditions such as climate, general surroundings (vegetation) and other animals present (pp. 588, 607).

*c.* Physiological animal geography offers a field for experimentation and observation which will have important bearing on human geography, sociology and psychology, and the general problems of biology and evolution (p. 609).

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# ON THE OLFACTORY ORGANS AND THE SENSE OF SMELL IN BIRDS

R. M. STRONG

*From the Hull Zoological Laboratory, University of Chicago*

TWO PLATES AND FOUR TEXT FIGURES

## CONTENTS

1. Introduction .....	619
2. Literature .....	620
A. General .....	620
B. Peripheral olfactory apparatus .....	621
1. The nasal chambers .....	621
2. The olfactory epithelium .....	622
3. The olfactory nerves .....	623
C. Central olfactory apparatus .....	623
1. The olfactory lobes .....	623
2. The olfactory fiber tracts .....	625
D. Observations of behavior .....	625
E. Reports of experimental studies .....	626
3. Methods and material .....	629
A. Morphological .....	629
B. Experimental .....	632
4. Morphological results .....	641
5. Results of experimental studies of the sense of smell in ring doves .....	646
6. Control experiments with white rats .....	650
7. Results of other experiments and observations .....	650
8. Conclusions .....	652
Bibliography .....	655

## 1. INTRODUCTION

The work described in this paper was done principally at the University of Chicago. About six years ago the writer became interested in the question of whether birds possess a sense of smell or not. The subject was assigned to a student for some preliminary study in the spring of 1905. During this time it became apparent that something more than simple direct tests would be

necessary, and the writer decided to study the problem by means of a labyrinth in which a demonstration of olfactory ability would require the association of an odor with the location of food. Such work was done mostly in the year 1907-8.

During the autumn of 1909, the writer enjoyed the privilege of studying the unique collection of bird brain material in the Senckenbergisches Neurologisches Institut at Frankfurt am Main, Germany. Though a large number of bird brain sections representing a good many species of birds were studied, no new facts of importance concerning central olfactory relationships were discovered from them. On careful examination of the literature it became apparent that there was need of a comparative study of the lobes and nerves, which could be done to advantage with the fine series of partly dissected heads in Professor Edinger's collection. These were placed at my disposal for further dissection and study. The nasal chambers also were studied.

The writer wishes to express his hearty thanks to Prof. Dr. Ludwig Edinger, the director of the Institute, for the opportunities afforded and for his helpful interest. Thanks are also due to Dr. W. M. Cooper of Frankfurt, to Dr. Priemal, the director of the Frankfurt Zoologischer Garten, to Prof. John B. Watson of Johns Hopkins University, and to Mr. W. H. Osgood of the Field Columbian Museum of Chicago for additional material and for courtesies received. Through the kindness of Mr. Seth Smith and Mr. R. I. Pocock, the privilege of making a test of the olfactory sense in *Apteryx* was enjoyed at the London Zoological Gardens. Assistance in the preparation of the drawings was received from Mrs. Strong.

## 2. LITERATURE

### *A. General*

During the early part of the last century, a spirited controversy was waged by a number of naturalists over the question of the existence of an olfactory sense in birds. So much evidence on the negative side was brought forth as to put the general occurrence of a sense of smell in birds in doubt ever since.

The presence of normal olfactory apparatus in birds has been recognized by a number of writers. In Apteryx, according to Parker ('91) and Owen ('71), the olfactory organs are relatively large for a bird. In other groups of birds the olfactory apparatus is generally recognized as small when compared with mammals, but varying in size. Thus Scarpa ('89), Schultze ('62), and others speak of well developed organs in the swimming birds, in the wading birds, and in the birds of prey. Bumm ('83), recognizes a relatively large olfactory apparatus in the swimming birds, but not in the birds of prey studied by him. The gallinaceous birds and the singing birds are described as having very much reduced olfactory organs.

### *B. Peripheral olfactory apparatus*

1. *The nasal chambers.* The nasal chambers of birds have been studied from various standpoints by a number of writers, including the following especially:

Beeker ('03) Common fowl (Gallus) and duck (Anas)	ogeranus, Psittacus, Picus, Caprimulgus, Podargus, Sturnus, Corvus, and other forms
Born ('79) Chick (Gallus)	Giebel ('76) Seventeen species of birds
Cohn ('02) Chick	Mihalkovics ('98) Gallus
Dieulaufé ('04 and '05) Paroquet, duck, turkey, dove, and vulture	Owen ('72) Apteryx
Exner ('72) Fowl, duck, dove, and some finches	Parker ('91) Apteryx
Ganin ('90) Eighteen genera of birds	Schultze ('62) Falco, Strix, Gallus, Columba, Anas and other birds
Gegenbaur ('73) Columba, Gallus, Meleagris, Anser, Buteo, Strix, Gyp-	

In general, two or three turbinals or conchae are recognized as occurring in the nasal chambers of birds. According to Gegenbaur the so-called superior or posterior concha is better named a 'Riech-hügel,' as, in the material he studied, it was found by him to be only an elevation or projection which did not possess the characteristic rolling of a true turbinal. Beeker ('03) supported him in this position. In some species Gegenbaur found even a 'Riech-hügel' lacking. The other turbinals are regularly designated as median or middle and inferior or anterior.

The turbinals of Apteryx are described by Parker ('91) as having an 'extreme complexity' (p. 49). In addition to the three turbinals already mentioned, he found 'anterior and ventral accessory turbinals.'

The absence of a posterior concha in the smaller species of birds was noted by Schultze ('62) and also by Giebel ('76). The latter considered this structure also lacking in *Corvus* and *Garrulus*. He found three turbinals in *Lanius excubitor*, however.

Jacobson's organ occurs in rudimentary or vestigial form according to Mihalcovics ('98), Ganin ('90), and Cohn ('02), in the embryo bird. It is lost during embryonic life, though the median portions of the ducts of the nasal glands are regarded as modified Jacobson's organs by Ganin and Mihalcovics.

2. *The olfactory epithelium.* In Apteryx, according to Parker, all of the turbinals, except the so-called ventral accessory, are covered with 'Schneiderian membrane' (p. 51). Owen, using a different terminology for the turbinals of Apteryx, also described an extensive distribution of olfactory nerve fibers in this bird both on all of the turbinals, excepting the 'anterior,' and on the septum narium.

In other birds studied, a much more limited distribution of the olfactory epithelium has been found. In the common fowl, according to Mihalcovics, the olfactory epithelium is limited to the posterior turbinal and to the adjacent wall of the nasal cavity up to the roof. A similar location was noted by Dieulafé ('04, p. 439). Preobraschensky ('92) found the olfactory epithelium limited to the posterior turbinal only, in the chick.

According to Schultze, the surface of the posterior turbinal may not always be entirely covered by olfactory nerve terminations. Doves were found to have the lower border free from olfactory epithelium. Gegenbaur believed that Schultze had the middle turbinal in mind, and the former writer says that doves do not have a posterior turbinal. Schultze states that in those singing birds which lack a posterior turbinal a very small part of the structure which corresponds to the middle turbinal receives olfactory nerve fibers. He also found the septum narium receiving olfactory nerve fibers, at least in those birds which have a posterior

turbinal. The structure of the olfactory epithelium has been most fully described by Schultze, though it has been studied by Exner and Disse.

3. *The olfactory nerves.* The olfactory nerves of birds have been given very little attention except from the standpoint of their development in the chick. They have been figured in drawings of the internal anatomy of the head by Scarpa for a few species of birds. There are also a few figures by Gadow ('91), in Bronn's Thier-Reich.

A single pair of so-called olfactory nerves are generally recognized as connecting the olfactory epithelium with the brain. (A more appropriate term would be 'olfactory root.' See Edinger, '08, p. 252.) However, in Apteryx, according to Owen, there are more than two. He says that "the olfactory nerves perforate the anterior and inferior wall of the rhinencephalic chamber by several foramina, but are closely invested and united by the neurilemma, especially along their upper surfaces, so as to appear for an extent of eight or nine lines, each as one large olfactory nerve." Parker ('91, p. 107), says that "the numerous olfactory nerves are given off from the ventral and anterior surfaces of the rhinencephal." The olfactory nerves of the vulture are stated by Owen ('66), to be larger relatively than those of the common turkey. They were found by Gage ('96) to be minute in the English or house sparrow.

The writer unfortunately has not had access to some of the old works on bird anatomy which may have had accounts of olfactory nerves.

The early development of these structures in birds has been studied by Cohn ('02), Disse ('96-'98) in the chick, goose, and duck, by Kölliker ('90), Marshall ('78), Preobraschensky ('92), in the chick, and Belogowy ('09), in the chick.

### *C. Central olfactory apparatus*

1. *The olfactory lobes.* The olfactory bulbs of many vertebrates are represented in birds, according to Edinger, by a *Formatio bulbaris* which covers the olfactory lobes except on their caudal dorsal borders. In the literature of bird brains, this com-

bination is usually designated as an olfactory lobe and will be so termed in this paper.

In Apteryx, the olfactory lobes are of considerable size according to the figures of Owen ('72, pl. xlv, fig. 2). This writer says (p. 383), that the "Rhinencephalon is as remarkable for its large size as is the mesencephalon for the smallness of its principal elements," in Apteryx. The optic lobes and nerves are small in Owen's figures. The only other reference to very large olfactory lobes in a bird is that of Klinekowström ('90), who figured and described large olfactory lobes for *Fulmarus glacialis*.

The olfactory lobes of a number of birds were studied by Bumm ('83) who noted that these structures are smaller in birds than in mammals. He states that the olfactory lobes are well developed in the swimming birds, moderately large in the marsh birds ('sumpf Vögel'), and much less developed in other orders. The ratios in weight of the olfactory lobes to the cerebrum are given for the 'Gans,' 'Schnepe,' and 'Bussard.'

Some statistics are given for the olfactory lobes in forty-two species of American birds, the majority being Passerine forms, by Turner ('91). Turner concluded that "there has been a gradual retrograde evolution of the avian rhinencephalon" ('91, p. 57). He observed that "as we ascend the scale, the olfactory lobes move caudad and become smaller," and he also noted that they are fused and almost completely imbedded in the 'prosencephalon' in the higher groups. In another article, Turner ('91b), stated that the high development of the sense of vision in birds has been accomplished at the expense of the olfactory sense.

The histological structure of the bird olfactory lobes has been described by Turner ('91), and by Pedro Ramon y Cajal. The latter's account was not accessible to the writer, and it has been necessary to depend upon the statement of Edinger ('03, p. 403) of Cajal's conclusions. The structure is stated to be of the same type as in other vertebrates except for being simpler.

References to the olfactory lobes have also been made by Carus ('14), Elliot-Smith ('95), Stieda ('69), Herrick ('93) Schüpbach ('94), and Kappers und Theunissen ('08).

2. *Olfactory fiber tracts.* Very little is known about the central relationships of the olfactory organs in birds. References are made to olfactory fiber tracts by Stieda ('69), Bumm ('83) Elliot-Smith ('95), Münzer und Wiener ('98), and Kappers und Theunissen ('08).

Edinger ('03) states that a basal bundle (*tractus bulbo corticalis*), consisting of a few fibers, passes into the brain base and is lost after a short course. Amputation of the olfactory lobes leads to degeneration of medullated fibers in the lobes only. According to the accounts of Edinger and Kappers, only a small number of olfactory fibers of the second order have been seen and these have not been satisfactorily traced. In the work of Kappers and Theunissen it is stated that olfactory fibers of the third order which connect the olfactory lobes with caudal non-cortical portions are more numerous. An olfactory cortex or hippocampus has not been demonstrated clearly.

#### D. *Observations of behavior*

A large number of miscellaneous field observations have been reported. As an illustration, an article by Rhoads may be cited. This observer states that while digging sweet potatoes in New Jersey, he noticed a luxurious growth of vines over a small mound in the field, and the potatoes dug at this place were unusually large. On inquiry, he found that a horse and a cow had been buried there during the previous winter. In the afternoon and during the following day, vultures came 'in scores', swooping to the ground about the mound. These birds continued to come 'for long after,' though not so numerous as at the time when the crop was plowed out. Rhoads could detect no 'taint' in the atmosphere, yet hundreds of vultures assembled 'from far and near.' He concluded that these birds were attracted by an olfactory stimulus.

According to Reeker ('99), a number of birds which were observed feeding on table scraps in a back yard declined to eat a potato which had been bitten by a cat. The author concluded that the potato was neglected because of an odor left by the cat.

Raspail ('99 and '01), credits birds with a very keen sense of smell. He gives a number of observations of occurrences in the field, and he reports various simple and uncontrolled experiments.

A caged condor was observed by Gill ('04) to become very much excited when, during the dissection of a rabbit, the strong odor of the abdomen escaped into the room in which the cage was placed. The operation is stated to have been carried on 'quite out of sight of the condor.'

The chances for error in the interpretation of this kind of evidence are so great that it has little value.

### *E. Reports of experimental studies*

Here are included accounts only of experiments conducted with critical care. The following studies were made by Audubon ('35):

1. An entire deer skin, including the hoofs, and provided with artificial eyes, was stuffed with dried grass, the whole being allowed to become 'perfectly dry.' The stuffed skin was exposed in a large field, and the observer concealed himself not far away. In a few minutes a vulture, soaring about, saw the deer skin and sailed down to it. The hide was torn open, and much grass was pulled out.

2. A large dead hog was hauled to a ravine and concealed by a covering of cane. As the weather was warm, the body became 'extremely fetid' in a couple of days. Dogs found the carcass and fed heartily upon it, but vultures sailing over from time to time did not find it.

3. A young pig was killed, and its blood was scattered about on the ground. The body was concealed by a covering of leaves. Vultures found the blood and followed it down the ravine to the body, which was then discovered and devoured.

4. Two young vultures were kept for some weeks in a cage where they became accustomed to receiving food. The birds were in the habit of hissing and gesticulating when they saw food approaching. However, when food, either fresh or putrid, was brought up to the immediate rear of the cage where the vultures could not see it, no excitement was shown.

Audubon quotes some experiments by Bachman from Loudon's Magazine of Natural History, 1838, as follows:

1. A dead hare, two dead birds, and a wheelbarrow full of offal from a slaughter house were deposited on the ground at the foot of Bachman's garden in South Carolina. A frame was raised above the pile at a distance of twelve inches from the ground, and this was covered with brush, allowing air to pass under freely. Though hundreds of vultures passed over in the next twenty-five days, none noticed the meat.

2. A coarse painting on canvas of a sheep skinned and cut open was placed on the ground, where it was noticed by vultures. They walked over the painting and tugged at it with their beaks. The painting was then placed within fifteen feet of the offal mentioned above, but the offal was not touched.

3. The most offensive portions of the offal were next placed on the ground, and these were covered by a thin canvas cloth on which were strewn several pieces of fresh beef. Vultures came and ate the beef, but they did not discover the offal beneath the canvas. A rent was then made in the canvas, whereupon the offal below was seen and eaten.

Negative results were also obtained by Darwin ('34), pp. 184-186), who experimented with a number of condors in a garden at Valparaiso, Chili. In his account Darwin says:

The condors were tied, each by a rope, in a long row at the bottom of a wall and having folded up a piece of meat in a white paper, I walked backwards and forwards, carrying it in my hand at the distance of about three yards from them, but no notice was taken. I then threw it on the ground, within one yard of an old bird; he looked at it for a moment with attention but then regarded it no more. With a stick I pushed it closer and closer, until at last he touched it with his beak; the paper was then instantly torn off with fury, and at the same moment, every bird in the long row began struggling and flapping its wings.

Darwin did not state whether the meat used in this experiment was entirely concealed by the wrapping paper until the package was torn open, nor did he indicate the opportunity for an odor to escape from the package. One is warranted in inferring from the account that the meat was detected before it was exposed to

view. The description of this experiment would suggest that the meat was finally smelled, though one may also infer that the olfactory sense was probably not extremely keen in these birds. Darwin himself concluded that "the evidence in favor of, and against, the acute smelling powers of carrion-vultures is singularly balanced," but he evidently believed that these birds find their food by sight.

Experiments of a different nature were conducted by Hill ('05) with a pair of turkeys. He employed a number of odors including many which should produce strong stimulation of nerves of general sensation, under the conditions of the experiments. Such substances as asafoetida, essence of anise, oil of lavender, valerianate of zinc, powdered camphor, chloroform, etc., were placed very near, or upon, one of two piles of food located in an enclosure into which the turkeys were admitted when they were to be fed. A number of trials were made to see whether the birds would make their choice of a pile of food because of the presence or absence of one of the odorous materials, but with negative results. No evidence of discrimination appeared, and even the fumes of prussic acid, though causing the bird to stagger, did not drive it from the first pile of food it happened to choose.

Laboratory experiments were conducted by Rouse ('05), who observed the respiratory movements of pigeons when in the presence of oil of bergamot and lily of the valley. No 'appreciable reactions' to these materials were noticed, but a 'slight sensitiveness was shown to asafoetida,' and 'marked reaction' was produced by turpentine and ammonia. The writer recognized, however, that the reactions obtained may have been due to other than olfactory stimuli.

On the other hand, evidence has been obtained by Beebe ('09) at Bronx Zoological Park, New York, that the turkey vulture has a sense of smell. The following is taken from his account (pp. 467-8) of experiments with turkey and black vultures:

Three boxes were placed on the ground some distance apart, and the birds fed for a few days in various parts of the cage. Then after several days of fasting, a piece of tainted meat was placed under the central box. Care was taken to go through the farce of placing something

under each box so that no visual hints of the location of the meat was conveyed. The vultures were very hungry, yet they did not leave their perches and come to the ground, although they had watched their keeper intently. He now re-entered and threw down one or two small bits of meat. Within a second or two, almost as the meat left the hand of the keeper, every vulture swooped to the ground and was hissing and struggling for a portion of the food. Twice the black vultures walked close about the meat box without appearing to notice the odor which was clearly perceptible, even to persons outside of the cage. A turkey vulture walked to leeward, instantly turned and made his way to the box, which he examined on all sides. He was soon joined by two others of the same species, and all three took up their stations close to the source of the odor. Soon two black vultures came up, apparently impelled more by imitation than by actual discovery of the smell. All five birds remained for a long time grouped close to the box, going to it now and then, and examining it carefully. Thus even in the turkey vulture the sense of smell is certainly not highly developed, and compared with the sense of sight, is defective indeed.

The experiments of Benham ('06), gave evidence that *Apteryx* has an acute sense of smell.

A preliminary statement of conclusions which were made from the experimental work described in this paper was published by the writer (Strong '08).

### 3. METHODS AND MATERIAL

#### A. *Morphological*

The olfactory lobes and nerves and the nasal chambers were studied in dissections of the bird head. As the olfactory lobes and nerves are always more or less imbedded in bone or cartilage and there is often considerable tough connective tissue directly over the lobes, dissection is not easy. A pair of small, pointed bone-forceps were found especially useful for the larger heads. For very small birds, a pair of scissors with curved points, dissecting needles, and a lens were employed. Measurements were made with the aid of a pair of dividers of the breadth of the cerebrum and of its length in the median line. The length of the olfactory nerve from the apex of the olfactory lobe to the nasal chamber and the diameter of the olfactory nerve were also determined. Of course all of these measurements were approx-

imate only, because it was impossible to select points for measuring with any precision. The olfactory nerve diameter was the most important of the measurements, and it could be determined with somewhat greater precision than the others. Nevertheless, the olfactory nerve cross section varies in form and size at different points, and a mean diameter for the median portion was consequently sought. The olfactory nerves in some species are flatter laterally than in others. Where they were very much flattened, lateral and dorso-ventral diameters were measured.

Photographs varying from one-half diameter to nearly natural size were made of the dissections in dorsal view. The heads were arranged so that the olfactory nerves were approximately at right angles to the axis of the camera. Some of the dissections were also photographed from the side to give a lateral view of the olfactory lobes and nerves. Prints enlarged two or three diameters were then made from the negatives of subjects which were selected for illustration in this paper. A magnification of three diameters was used only for a few small birds. Most of this work was done with the Edinger drawing apparatus. When this apparatus was properly adjusted for an exposure, and before a print holder was inserted, a sketch was made of the image projected on drawing paper. This sketch gave the general outlines for the making of a drawing. References were made frequently to measurements and to the enlarged prints in the preparation of the drawings. Three methods were thus available for securing accuracy in the drawing of the illustrations. Most of the brain mass has been included in order to show the relative size of the olfactory lobes.

Heads of the birds in the following list were dissected and studied: The classification used in Sharpe's Catalogue, ('74) and Handlist, ('99) is employed here and the orders are given so that the taxonomic distribution of the forms studied may be seen at a glance. The Sharpe Catalogue nomenclature has been followed consistently because at present there is no other work so representative of the birds of the world. As many of the specimens in the Edinger collection bore other names, they also are mentioned after the Sharpe Catalogue name in parentheses.

This material represents twenty-seven out of thirty-five orders of existing birds. The species which were studied in Professor Edinger's laboratory are indicated by an asterisk.

## SUB-CLASS RATITAE

- Order 1. Rheiformes:* \**Struthio camelus*, Linn. Ostrich.  
 \**Rhea americana*, Linn. Common rhea. *Order 3. Casuariiformes:*  
 \**Dromaeus novae-hollandiae*, Lath. Emeu.  
*Order 2. Struthioniformes:*

## SUB-CLASS CARINATAE

- Order 1. Tinamiformes:* \**Nothura maculosa*, Temm. (Rhy-chotus). Spotted tinamou.  
*Order 2. Galliformes:* \**Crax carunculata*, Temm. Yarell's curassow.  
 \**Lyrurus tetrix*, Linn. Black grouse (Tetrao).  
 \**Caccabis petrosa*, Gm. Barbary partridge.  
 \**Perdix perdix*, Linn. Common partridge.  
 \**Gennaeus nycthemerus*.<sup>1</sup> (*Euplocamus argentatus*). Silver pheasant.  
 \**Gallus gallus*, Linn. Common fowl.  
 \**Polyplectrum chinquis*, P. L. S. Mull. Gray peacock-pheasant.  
*Order 5. Columbiformes:* \**Columba livia*, Bonn. Domestic dove.  
 \**Turtur risorius*, Linn. Ring dove.  
 \**Goura coronata*, Linn. Crowned pigeon.  
*Order 7. Ralliformes:* \**Rallus virginianus*, Linn. Virginia rail.  
 \**Fulica atra*, Linn. European coot.  
 \**Fulica americana*, Gm. American coot.  
*Order 11. Sphenisciformes:* \**Spheniscus demersus*, Linn. Cape penguin.  
*Order 12. Procellariiformes:* \**Fulmarus glacialis*, Linn. (Procellaria). Fulmar.  
*Order 14. Lariformes:* \**Hydrochelidon surinamensis*, Gm. Black tern.  
 \**Sterna fuliginosa*, Gm. Sooty tern.  
 \**Anous stolidus*, Linn. Noddy tern.  
 \**Larus ridibundus*, Linn. Black headed gull.  
*Order 15. Charadriiformes:* \**Belanopterus cayennensis*, Gm. (Vanellus). Cayenne lapwing.  
 \**Pavoncella pugnax*, Linn. Ruff. (Machetes).  
 \**Scolopax rusticola*, Linn. European woodcock.  
*Order 16. Gruiformes:* \**Anthropoides virgo*, Linn. (Grus). Demoiselle crane.  
 \**Eurypyga helias*, Pall. Sun bittern.  
*Order 18. Ardeiformes:* \**Theristicus melanopsis*, Gm. (Ibis). Black-faced ibis.

<sup>1</sup> The identification of '*Euplocamus argentatus*' with any name in the Sharpe nomenclature was difficult. On consultation of other works it seemed probable that the bird is *Gennaeus nycthemerus* in the Sharpe system.

\**Platalea leucorodia*, Linn. White spoon-bill.

\**Pseudotantalus leucocephalus*, Gm. (*Tantalus*). White headed ibis.

\**Leptotilus crumeniferus*, Less. African adjutant.

\**Tigrisoma lineatum*, Bodd. (*T. brasiliense*).

\**Nycticorax cyanocephalus*, Mol. Night heron.

Order 19. *Phoenicopteriformes*:

\**Phoenicopterus roseus*, Pall. (*P. antiquorum*.) European flamingo.

Order 20. *Anseriformes*:

\**Plectropterus niger*, ScL. Black spur-winged goose.

\**Anser anser*, Linn. Domestic goose.

\**Anas boschas*, Linn. Domestic duck.

Order 23. *Pelecaniformes*:

\**Phalacrocorax carbo*, Linn. Cormorant.

\**Plotus anHINGA*, Linn. Snake bird.

\**Sula bassana*, Linn. Gannet.

\**Pelecanus rufescens*, Gm. Red-backed pelican.

Order 24. *Cathartidiformes*:

\**Catharistes urubu*, Vieill. (*C. atrata*). Black vulture.

\**Cathartes aura*, Linn. Turkey vulture.

Order 25. *Accipitriformes*:

\**Circus aeruginosus*, Linn. The Moor buzzard.

\**Accipiter nisus*, Linn. European sparrow hawk.

\**Buteo buteo*, Linn. European buzzard.

\**Circætes gallicus*, Gm. Serpent eagle.

Order 26. *Strigiformes*:

\**Bubo ignavus*, Forst. Eagle owl.

Order 28. *Psittaciformes*:

\**Chrysotis auripalliata*, Less. Golden-naped Amazon parrot.

\**Chrysotis brasiliensis*, Linn. Red-tailed Amazon parrot.

Order 31. *Coccyges*:

\**Coccyges erythrophthalmus*, Wils. Black-billed cuckoo.

Order 32. *Scansores*:

\**Rhamphastos cuvieri*, Wagl. Cuvier's toucan.

Order 33. *Piciformes*:

\**Gecinus viridis*, Linn. Green woodpecker.

Order 36. *Passeriformes*:

\**Sylvia atricapilla*, Linn. Blackcap

*Lanius migrans*, Linn. Migrant

shrike.

\**Motacilla alba*, Linn. White wagtail.

\**Coccothraustes coccothraustes*, Linn. Hawfinch.

\**Serinus serinus*, Linn. Serin finch.

\**Sturnus vulgaris*, Linn. Starling.

\**Corvus corax*, Linn. Raven.

\**Corvus corone*, Linn. European carrion crow.

*Corvus brachyrhynchus*, Brehm.

American crow.

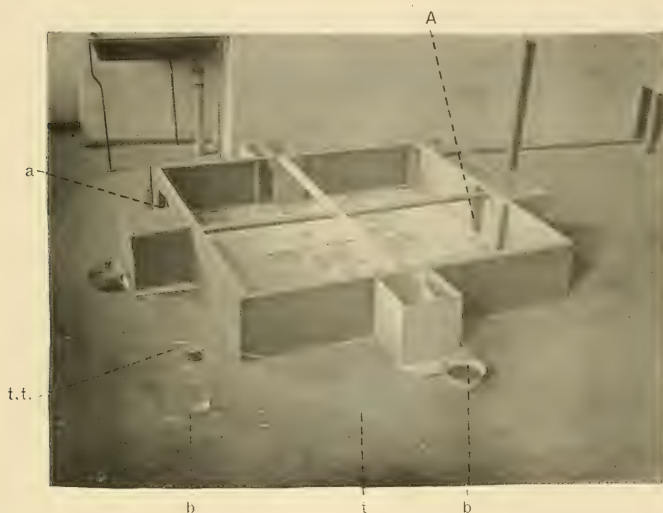
\**Garrulus glandarius*, Linn. European jay.

*Cyanocitta cristata*, Linn. American blue jay.

## B. Experimental

The first attempts by the writer to determine whether an olfactory sense exists in birds or not were simple experiments with such substances as cedar oil, asafoetida, oil of bergamot, clove oil, and hydrogen sulphide. The odorous material, placed upon rags and filter paper or in bottles, was usually held within a few feet or inches of the bird's head. Several nestling and adult ring

doves, two young crows, some common fowls, turkey vultures, and a paroquet were used as subjects for the tests. As these experiments proved to be worthless, it appeared to be necessary to develop more elaborate methods for distinguishing possible responses to olfactory stimuli, and a labyrinth in which the bird



Text fig. A Labyrinth without cover; *d*, entrance to chamber A; *b*, liter wash-bottle; *b'*, small bottle which contained the odorous material and was located here when the food box was in the position which is indicated in fig. C; *t*, glass tube which connected odor bottle with the wash bottle; *tt*, tube leading to two small bottle connections; *d*, entrance to main enclosure from cage.

would have the opportunity of finding its food was devised. This apparatus, shown in figs. A and B, included a central area five feet wide and ten inches high, with four accessory chambers, each of which opened into the central enclosure at the middle of a side. The chambers were ten inches square and so arranged that a bird in the central enclosure could not see food placed at *f.b.*, fig. C.

The dimensions given above were chosen mainly in order that the apparatus might be accommodated to the size of the room available for the experiments, and they seemed to be suitable for the work with ring doves as the experiments proceeded.



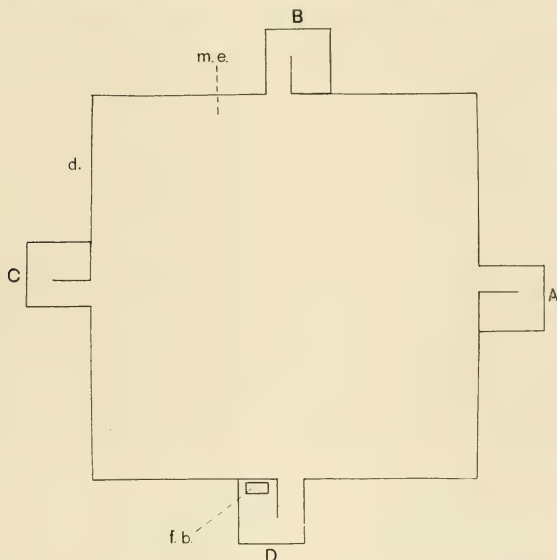
Text fig. B Labyrinth with cover (*c*) in place; *f*, funnel of exhaust; *p*, Richard's air pump (two air pumps were used at this point in the oil of bergamot series).

The control of air currents naturally demanded an indoor location for the apparatus.

In order to reduce the danger of general diffusion of the odor, an air exhaust was arranged. A funnel with a diameter of  $6\frac{1}{2}$  inches at the larger end was mounted with the small end up at the center of the cover to the main enclosure, and this was connected with a Richards<sup>2</sup> air pump which was attached to a water

<sup>2</sup> During most of the experiments with oil of bergamot, two of these pumps were used with a **T** connection in order to accelerate the removal of diffused odor from the central enclosure.

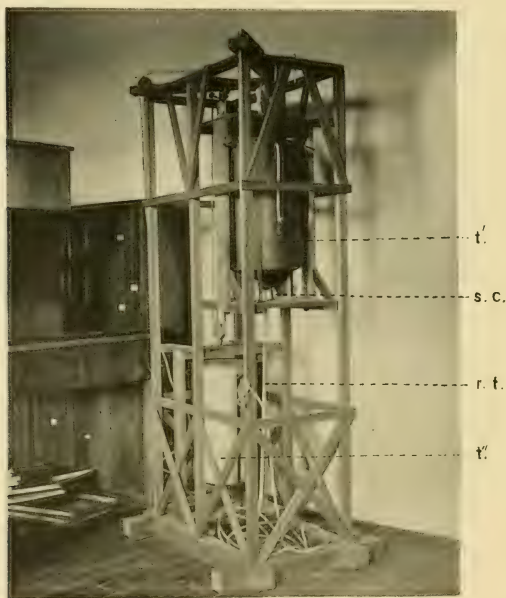
tap (fig. *B*). Gentle air currents were forced into each compartment through glass tubing arranged as seen in fig. *B*. The air currents were produced by a machine originally constructed for injecting blood vessels. In this apparatus (fig. *D*), water was passed from one closed tank into another by elevating the filled tank above the empty one. The two tanks were connected



Text fig. C Diagram of labyrinth; *A, B, C, D*, chambers; *d*, position of entrance to main enclosure (*m. e.*) from cage; *f. b.*, food box.

by rubber tubing (*r.t.*, fig. *D*). The air displaced by the entering water in the lower tank was allowed to escape through stop cocks into two glass tubes. Each of these tubes communicated with a wash bottle (one-litre size) which was about three-fourths full of water. From the wash bottles, the air emerged through **T** tubes (fig. *A*, *t.t.*) and was conveyed to 125 cc. bottles (fig. *A*, *b'*) opposite the four chambers of the labyrinth. These smaller

bottles were mounted in dishes which contained wax in order to prevent them from being upset. The glass tubing used was of uniform diameter, and the ends which projected inside the chambers were carefully rounded so that they might present no discernible differences in appearance. This tubing had an inside



Text fig. D; Air pressure apparatus (not designed by the writer); *t'*, upper tank; *t''*, lower tank; *r. t.*, rubber tube which connected the tanks; *s. c.*, stop cock.

diameter of about 5.5 mm. One only of the small bottles was used to contain the odorous material, and it was disconnected after each set of experiments to be put away until the next series of experiments were begun. Food (a mixture of canary seed, millet, and wheat with a small admixture of ground charcoal and oyster shells) was placed for each series of four experiments in

one of the four chambers (fig. *C, f.b.*), and the odor bottle was connected with the chamber which contained the food, where it replaced the bottle located there in the previous series of experiments (fig. *A, b'*). Newspapers were laid underneath the entire labyrinth to prevent the birds from soiling the floor.

After the apparatus had been inspected to insure its being ready, the air pumps were started by turning on water at *P*, fig. *B*. The cage containing the bird was placed with the door against the entrance to the enclosure (fig. *A, d*); the cage door was then raised, allowing the subject to enter. It was not found practicable, though desirable, to place this entrance at the center because there was greater need of room for the exhaust funnel at that point. Immediately after the dove was admitted, air pressure was started by turning a cock at *s.c.*, fig. *D*. This order of procedure insured the entrance of the odor into the enclosure by the time the bird started to look for food, and the postponement of turning on the air pressure until just after the bird had entered decreased the danger of a general diffusion of the odor in the central enclosure during the time which was ordinarily occupied by the subject in hunting for the food.

When the bird reached the seed, the air pressure was cut off to avoid unnecessary diffusion of the odor, but the exhaust was kept going until the last of the birds used in each set of experiments had found its food.

When the last bird had finished feeding and had been removed to its cage, the entire labyrinth was elevated several inches above the floor for ventilation; a window and a door to the room containing the apparatus were kept open for a short time at least during the ventilation.

In order to determine so far as possible, the extent of the space in the main enclosure where the odors which were employed in the experiments might be expected to be perceptible, various tests were made. Strips of litmus paper about eight inches long were hung from standards placed at thirty-six approximately equal points inside the main enclosure. These strips were all dipped in distilled water just before air pressure was turned on. Ammonia water of several dilutions was placed in a bottle which was used

in place of the odor containing bottle. When a relatively strong solution of ammonia was used, all of the litmus paper strips turned blue in the course of a few minutes, those in the corners changing last. When solutions produced a diffusion of ammonia gas which seemed to be at all comparable in strength with the odor of oil of bergamot, so far as such a comparison could be made, a semi-circular area of diffusion was indicated by the litmus paper. The radii of this area converged at the entrance of that chamber where the ammonia gas emerged, and its front extended out to the region of the exhaust funnel. The writer stretched himself on the floor inside the enclosure and attempted tests of the odor diffusion with his own olfactory organs. Musk and violet sachet powder were smelled with difficulty and only at the entrance where these odors were emerging. The odor of oil of bergamot was detected as far as eighteen inches from the point of emergence. The odor was not strong beyond a foot or less from the entrance of the chamber from which the odor was emerging.

Of course these experiments afford only indirect evidence concerning the size and form of the area in which the odor might be expected to be effective. However, the behavior of the birds and of some rats in the enclosure, when considered in connection with the tests mentioned above, have convinced me that the odor localization was sufficient to enable an animal which would be capable of odor discrimination to determine the compartment from which the odor emerged in the experiments. White rats appeared to have no difficulty in locating the source of the odor.

*Choice of subjects.* It was obviously necessary in using such apparatus that only tame and tractable birds be employed. Of various species which were considered, ring-doves seemed to be most suitable. The writer had on hand a number of hybrids between the white and blonde ring-doves *Turtur alba* and *T. risorius* which were unusually tame; four vigorous males were chosen. Unfortunately, the habits of ring-doves do not suggest that they have any use for a sense of smell. Their food consists mostly of seeds which have practically no odor for the human olfactory sense. Nevertheless, their great docility, convenient size, and adaptiveness to cage life made them preferable, in the

writer's judgment, to less tractable birds whose habits might suggest greater olfactory possibilities. During the series of experiments there were many occasions when even these birds were none too manageable.

*Choice of odor.* An ideal odorous material for these experiments should be associated with the natural food-seeking habits of the subject. It should also stimulate only the olfactory sense organs. Unfortunately, the first condition could not be realized, and the second could not be established with absolute certainty. A careful consideration of food materials did not suggest anything under practicable conditions which would supply a strong odor as measured by the human olfactory organs. No odorous material of any kind could be found where the possibility of other than olfactory stimulation could be known to be absolutely eliminated. It was therefore necessary to consider odors which do not have any known relationship to the experience of ring-doves.

The following were selected as being the least objectionable: animal musk, violet-sachet powder, and oil of bergamot. Eau de cologne was used for the first series of experiments, but it was finally abandoned as the writer became impressed with the possibility of alcohol stimulation by the alcohol contained in this compound. Animal-musk and violet-sachet powder when they were not dissolved in alcohol produced odors so weak that they were employed only a short time. A strong odor seemed desirable because these birds could not be assumed to have so keen a sense of smell as even man possesses. Oil of bergamot was finally chosen for the larger portion of the work because it seemed to combine strength with freedom from at least apparent danger of there being other than olfactory stimulation. In the absence of knowledge concerning the effects of odorous materials upon the dove's sense organs, the sensations of the experimenter could be the only guide in choosing an odor. It may be objected that when held very near the nose, oil of bergamot produces almost painful sensations, which may involve tactile endings. Under the conditions of the experiments, however, the stimulation of olfactory endings was mild for the writer and there were no suggestions of any other stimulation.

*Management of birds.* Before the taking of records was begun, the doves were trained to look for food without the use of any odor. They were induced to enter a chamber by a trail of seed which led to the food box. In the course of a week, they lost their fear of entering the chambers, and it was found practicable to work with them twice a day.

The doves were kept in cages which stood in the room containing the apparatus. Their view of the apparatus was cut off by a screen, though this precaution was probably unnecessary. Even when the bird had been placed inside the main enclosure, on several occasions, before the seed box had been put in place, it gave no evidence of having profited by the opportunity it had had to see the location of the food, but went through the usual search for the proper chamber to enter. Thus, on one occasion, a dove after such an opportunity went to all three of the empty chambers before it entered the one containing the food.

Each bird was allowed enough time to eat all the food it wished. It then almost invariably returned to the main enclosure, and was driven out through the entrance *d*, fig. *A*, into a cage which had been placed alongside. After a few weeks, the removal was easily accomplished, for the doves became accustomed to passing out when a stick was waved over the apparatus.

*Records.* The significant movements of the birds were recorded usually by means of symbols. The sign = was used to indicate that the dove entered a chamber so far that it should have been able to see whether food was present or not. The simple approach of a bird to or within a few inches of the opening of a chamber without an entrance which would be complete enough to enable it to see whether food was present or not, was designated by the sign -. Thus the form 30 -  $B = C = A$  indicated that dove No. 30 in the writer's breeding register went to the opening of chamber *B* but did not enter. It then turned and entered chambers *C* and *A*, the latter containing the food. Notes were made of interesting or unusual variations in the behavior of the birds.

## 4. MORPHOLOGICAL RESULTS

The anatomical studies made by the writer have given evidence that (1), the olfactory organs of birds are of too great size to be set aside as non-functional, but that (2) there is a tendency in the bird series towards retrogression of these organs. Various types of olfactory lobes and nerves have been figured in plates 1 and 2, and measurements for some of the birds studied have been given in table 1.

In most of the orders, the olfactory lobes usually have essentially the form found in the doves and in the gallinaceous birds. They are so similar in the representatives of these two groups which were studied, that only one form has been chosen for illustration, and that is the species used by the writer in his experimental work (fig. 4). The so-called olfactory nerves usually leave the olfactory lobes in more or less close contact with each other, and they diverge gradually at some distance from their proximal ends as in fig. 4; but in some cases they are widely separated (figs. 1, 8, 9, 13, 16, and 17). In table 1, some variations have been noted under the head of remarks. The length of the olfactory nerves and their degree of separation seem to be adapted to the form and arrangement of other structures in the head, and with no functional significance that could be discovered. Olfactory nerves, so called, were found in all of the material studied except for *Dromaeus*, *Spheniscus*, and *Fulmarus*. In these three birds, the olfactory lobes have their anterior ends in contact with the nasal capsules, though *Dromaeus* may possibly be said to have very short olfactory nerves.

In no case was more than one pair of olfactory nerves found except possibly in *Rhea*. Unfortunately, the available material was not in a condition to give a satisfactory dissection for this species.

The most interesting olfactory organs in some respects were those found in *Dromaeus*, *Fulmarus*, *Catharistes*, *Cathartes*, and the *Corvidae*. In *Dromaeus* and in *Fulmarus*, the olfactory lobes are notable for their relatively great size (figs. 2, 3, 5, and 6). It will also be observed that there is a constricted connection of

TABLE 1

Measurements are in millimeters. Unless otherwise stated in the column headed remarks, the olfactory lobes and nerves do not vary significantly in their size, form and arrangement from those of the doves and the gallinaceous birds. The species which appear in plates 1 and 2 have not been included here.

SPECIES	OLFACTORY NERVE		CEREBRUM		REMARKS
	Length	Diameter	Length	Breadth	
<i>Rhea americana</i> ....	9.0+	0.7	19.5	34.0	Lobes rather large and resembling those of <i>Dromaeus</i> slightly. Turbinals elaborate
<i>Crax carunculata</i> ...	11.5	1.15	20.0	29.0	Olfactory lobes long
<i>Lyrurus tetrrix</i> .....	12.0	0.5	14.0	23.0	
<i>Perdix perdix</i> .....	8.0	0.4	12.5		
<i>Gallus gallus</i> .....	12.0	0.7×1.0	14.0	22.5	
<i>Columbia livia</i> .....	10.0	0.6×.75	12.0	20.0	
<i>Goura coronata</i> .....	14.0	0.8	16.5	28.0	
<i>Fulica atra</i> .....	5.0	1.0	17.0	22.5	Olfactory lobes long as in duck
<i>Anous stolidus</i> ...	8.5	0.5×0.7	18.5	11.5	
<i>Larus ribidundus</i> ..	8.0	0.7	12.0	24.0	
<i>Belanopterus cayennensis</i> .....	11.0	0.4	12.5	20.0	
<i>Scolopax rusticola</i> ..	12.2	0.8	15.0	20.0	See next page.
<i>Anthropoides virgo</i> ..	0.8	0.1	19.0	30.5	
<i>Eurypyga helias</i> ....	9.0	0.4×0.8	12.5	19.2	Nerves very close together throughout
<i>Theristicus melanopsis</i> .....	9.0	1.0	22.5	35.0	Lobes relatively small
<i>Platalea leucorodia</i> ..	8.0	12.0	27.5	34.0	Lobes small and between anterior ends of hemispheres; ophthalmic branch of trigeminus very large
<i>Tigrisoma brasiliense</i> .....	15.5	1.2	18.0	29.0	
<i>Nycticorax cyanocephalus</i> .....	14.0	1.0	19.0	29.0	
<i>Anser anser</i> .....	7.0	1.5	33.0	56.0	Lobes long
<i>Anas boschas</i> .....	12.5	1.2	20.0	26.0	Lobes long
<i>Phalacrocorax carbo</i> .....	9.5	1.0	23.5	29.5	Nerves relatively small
<i>Plotus anHINGA</i> .....					Lobes relatively small
<i>Sula bassana</i> .....	17.0	0.7×1.2	24.5	37.2	Lobes have peculiar ventral position

TABLE 1 (continued)

SPECIES	OLFACTORY NERVE		CEREBRUM		REMARKS
	Length	Diameter	Length	Breadth	
<i>Rhamphastos cuculieri</i> .....	6.0	0.5	19.0	29.0	Lobes small; nerves wide apart; no external nares; nasal chamber very short
<i>Gecinus viridis</i> .....	9.5	0.3	19.0	23.0	Lobes very small
<i>Pelecanus rufescens</i>					Lobes small
<i>Circus aeruginosus</i> ..	10.0	0.5×0.8	18.5	32.0	
<i>Accipiter nisus</i> .....	9.5	0.6—	14.0	24.0	
<i>Buteo buteo</i> .....	12.0	0.5×1.1	19.0	35.0	Lobes small and short
<i>Bubo ignavus</i> .....		0.8	21.0	41.5	Lobes not studied successfully
<i>Sylvia atricapilla</i> ..	5.5	0.2	9.0	12.5	Lobes and nerves very small
<i>Sturnus vulgaris</i> ....	5.5	0.3	14.0	20.	Lobes small; nerves separated at post. ends.
<i>Garrulus glandarius</i>	9.0	0.2×0.4	27.5	25.5	Lobes minute and fused; olf. nerves very slender

the olfactory lobes with the brain in *Dromaeus*. Such a condition was found in less conspicuous form in specimens of the order. *Charadriiformes* which were examined by the writer, and it also appears in a figure of the brain of the American woodcock, another member of this order, (Herrick, '93, pl. 26). The writer regrets that he has not had opportunity to study the *Dromaeus* material histologically to note the relations of the formatio bulbaris to the olfactory lobe proper. There was evidence obtained by dissection that there is a comparatively rich innervation of the relatively complicated posterior turbinals, by olfactory fibers. The surface of the middle concha is also elaborate, as can be seen in the posterior portion which has been included in fig. 2, and it seems quite possible that the middle concha in *Dromaeus* also receives olfactory fibers.

Though the relatively huge olfactory lobes of the fulmar have been described by Klinekowström, they were not adequately illustrated in the figure which accompanied his account, and the nasal chambers were not included. It has therefore seemed desir-

able to the writer that a more complete illustration be published as has been done in fig. 5. The turns of the posterior turbinal may be seen at (*d*) where openings have been made in the preparation. The extensive rolling of this turbinal and the large size of the olfactory lobes in this species seem very significant, and the writer regrets that he has not had time to study the sense of smell in the living fulmar.

As the fulmar is one of a group of birds which are characteristically seagoing animals and which are in the habit of taking long trips over the sea, it is natural to suspect that these large olfactory lobes might be used as orientation organs if not serving for the sense of smell, (Cyon, '08). With this idea in view, the writer wrote to Prof. John B. Watson, of Johns Hopkins University during the early summer of 1910, while Professor Watson was conducting studies of orientation in the noddy and sooty terns at the Dry Tortugas Islands. Dr. Watson had discovered that these birds are able to find their way home over a presumably unknown area of the sea when taken from their nests for a distance of at least 850 miles, (Watson '08). Material of both the noddy and sooty terns was kindly furnished by Professor Watson. The olfactory lobes and nerves were both found to be relatively small even when compared with those of birds with olfactory organs of moderate size. The diameter of the olfactory nerves is given in table 1. In this connection it is interesting to note that Professor Watson ('10) conducted experiments in which individuals of both species had their external nares closed with wax, but the ability of the birds to find their way at sea was apparently not disturbed.

Although the crows and ravens have often been credited with a keen sense of smell, the olfactory lobes and nerves of the specimens examined were found to be surprisingly minute (fig. 17). In all of the Corvidae material studied, this condition prevailed. These structures were found with some difficulty lying deep in the interorbital space. As the Corvidae have been considered by some writers as standing at the top of the bird series, it is unusually interesting that the olfactory organs are smallest in this group. The minuteness of the olfactory lobes and nerves

argues for the greatest reduction of the sense of smell in this family.

The olfactory lobes of the two parrot heads which were dissected were found to be so merged with the fore brain lobes as to be undefinable, (fig. 13). Apparently the organs of smell are not well developed in these birds. The representatives of the Passerine birds studied were all found to have extremely small olfactory lobes and nerves, as has been the observation of otherwriters. The condition shown in fig. 16, is characteristic for the finches.

The writer agrees with Scarpa ('89), Schultze ('62), and Bumm ('83) in finding olfactory organs of good size in the swimming birds. These organs are also fairly large in the shore birds (Charadriiformes). The olfactory lobes and nerves of the birds of prey have, on the other hand, been found to be of relatively moderate size, thus confirming Bumm's observation. In fig. 12, a peculiarly fused and elongated condition of the olfactory lobes has been illustrated. In the specimens of the other birds of prey which were studied, these organs were more or less similar to those of the doves.

On comparison of all the available material, the olfactory organs are found to be, in general, largest in the so called lower groups and progressively smaller in the higher orders. The sense of smell has evidently been disappearing in birds with the great development of the sense of vision. It seems not at all improbable that the sense of smell may be practically lost in the Passerine birds.

The ophthalmic branches of the trigeminus after emerging from the orbits usually cross the olfactory nerves dorsally near their distal ends. Their course is often so close to that of the olfactory nerves at this place as to make it easy to confuse the trigeminus with the olfactory nerve. In the figures, the trigeminus nerve branches have been drawn slightly separated when desirable for the sake of clearness. In those forms where the trigeminus branches to the upper mandible were found to be especially large, the olfactory lobes and nerves were also of good size as a rule, and it seems not impossible that they may function in the feeding operations of birds like the ducks, the flamingos, etc., in more or less intimate relationship with the trigeminus.

# 5. RESULTS OF EXPERIMENTAL STUDIES OF THE SENSE OF SMELL IN RING DOVES

The methods which were employed in this work have been described on pp. 632-9, where it will be noted that a labyrinth was used, and the birds were compelled to find their food in one of four chambers which were selected by the experimenter at random.

If the doves had entered the chambers entirely in hit or miss fashion with no clues or habit preferences which would influence their choice of a chamber, they would be expected, according to the law of error, to distribute their first choices equally among the four chambers, and the compartment which contained the food would be entered first 25 per cent of the time. What actually happened is shown in the following tables:

TABLE 2

*Cologne series*

No. 62, 19 per cent; No. 30, 28 per cent; No. 92, 33½ per cent; No. 24, 24 + per cent

TABLE 3

*Musk series*

No. 62, 20 + per cent; No. 30, 28.9 + per cent; No. 92, 31 per cent; No. 24, 25 per cent

TABLE 4

*Violet sachet powder series*

No. 62, 50 per cent; No. 30, 40 per cent; No. 92, 28 per cent; No. 24, 75 per cent

TABLE 5

*Oil of bergamot series*

No. 62, 37 + per cent; No. 30, 31 + per cent; No. 92, 36 + per cent; No. 24, 47 per cent

It will be seen in the table that when cologne and musk were used, the percentages of correct first entrances were not significantly different from what might be expected according to the law of error. With oil of bergamot, however, the percentage is

notably large for all four birds. This is especially true of No. 24, a bird which gave other suggestions of discrimination in its behavior than are indicated in the tables. The percentages are also large in the violet sachet powder series, but not much significance can be attached to this fact because of the small number of experiments in this series.

It is of course conceivable that the food itself might have had an odor for the doves, even though none was apparent to the writer. However, if there had been any olfactory stimulation by the food, all four of the birds probably would have shown similar responses to the stimulus. That failure to find the food through a hypothetical odor from the food material was not due to disturbances produced by the odors which were employed in the experiments, was indicated in a short series of control experiments when no odors were used. In this series, the food was found entirely by chance, apparently.

There was an unfortunate tendency for the doves to enter chamber A first by habit. After this compartment had been entered, if it was empty, they would go through the usual hunt for the food and would ordinarily show no further tendencies to enter a second chamber *by habit*. The extent to which the birds made their first entrance *by habit* is indicated in the following tables.

Tables showing the number of times each chamber was entered first:

TABLE 6

*Cologne series*

No. 62	A, 8; B, 14; C, 6; D, 5
No. 30	A, 13; B, 1; C, 2; D, 7
No. 92	A, 18; B, 0; C, 4; D, 8
No. 24	A, 12; B, 4; C, 8; D, 5

TABLE 7

*Musk series*

No. 62	A, 11; B, 15; C, 14; D, 5
No. 30	A, 32; B, 4; C, 3; D, 2
No. 92	A, 38; B, 4; C, 3; D, 5
No. 24	A, 23; B, 8; C, 4; D, 5

TABLE 8

*Violet sachet powder series*

No. 62	A, 0; B, 2; C, 1; D, 1
No. 30	A, 3; B, 0; C, 0; D, 2
No. 92	A, 6; B, 0; C, 0; D, 1
No. 24	A, 2; B, 2; C, 0; D, 0

TABLE 9

*Oil of bergamot series*

No. 62	A, 35; B, 73; C, 54; D, 24
No. 30	A, 109; B, 17; C, 8; D, 12
No. 92	A, 94; B, 31; C, 12; D, 39
No. 24	A, 31; B, 42; C, 24; D, 44

In those experiments where *A* was not the first chamber entered, the percentage of correct first entrances made by No. 30 in the oil of bergamot series was 41, and 44 per cent for dove No. 92.

It will be seen that doves Nos. 30 and 92 both entered chamber *A* first a very large number of times. This habit became so confirmed in No. 30 that experiments with this bird were finally discontinued. Attempts were made to break up the habit, but no success was obtained, except when the food was placed regularly at one of the three other chambers. This could not be done much, of course, without seriously affecting the results of the experiments. It was desirable that there should be little difference in the number of times each chamber was used for food. It will be noticed that dove No. 62 did not develop such a habit and that No. 24 did not exhibit the tendency in the oil of bergamot series.

In order to test the possible odor discrimination of the doves after they had made one mistake, the means of errors made by the birds were calculated. If, for instance, the birds entered the chambers at random and did not go into any single chamber more than once, they would, in a sufficiently large series of experiments, be expected to have a mean of errors approximating 1.5. As a matter of fact, they often entered an empty compartment more than once before finding the food. Thus, on one occasion, No. 92 entered chambers *B* and *C* each three times before

finding the food at A. In this case *D* was entered once. The record of this result was written as follows: No. 92 = B = D = C = B = C = B = C = A. This repetition of errors increased the size of the mean appreciably, at first, in the cologne series. In the following tables it will be seen that the means were significantly small when oil of bergamot was used, in spite of the fact that errors were repeated occasionally.

TABLE 10  
*Cologne series*

BIRD	MEAN	PROBABLE ERROR OF MEAN
62 .....	1.5483—	0.179±
30 .....	1.58	0.1578±
92 .....	1.7083+	0.182±
24 .....	1.9689+	0.189±

TABLE 11  
*Musk series*

BIRD	MEAN	PROBABLE ERROR OF MEAN
62 .....	1.5	
30 .....	1.447	
92 .....	1.549	
24 .....	1.6	

TABLE 12  
*Oil of bergamot series*

BIRD	MEAN	PROBABLE ERROR OF MEAN
62 .....	1.18+	0.058±
30 .....	1.369+	0.0703±
92 .....	1.193+	0.0625±
24 .....	0.922—	0.0663±

The totals of trials given the doves for different odors will be found in the following table:

TABLE 13

Bird.....	62	30	92	24
Cologne.....	31	25	30	29
Musk.....	44	38	51	40
Violet sachet powder.....	4	5	7	4
Oil of bergamot.....	186	146	176	141

## 6. CONTROL EXPERIMENTS WITH WHITE RATS

A pair of rats were used as a test of the efficiency of the apparatus. They gave the following results when oil of bergamot was employed, the conditions being those which were furnished the ring doves.

	PERCENTAGE	MEAN	TOTAL NUMBER OF TRIALS
Male rat.....	0.62	0.406+	59
Female rat .....	0.71	0.316+	60

It is the writer's opinion that the rats found their food usually, if not always, when not by accident, through an association of the odor of oil of bergamot with the location of the food. In a short series of trials which were made without any odor, the rats appeared to find the food (sunflower seed) by the method of trial. That these keen scented animals made so many mistakes is probably explained by their tendency to enter the first chamber they came to and sometimes the next in order before they made any attempt to localize the source of the odor of oil of bergamot which was all of the time entering the enclosure.

## 7. RESULTS OF OTHER EXPERIMENTS AND OBSERVATIONS

The writer spent about ten weeks of the winter of 1906 in Florida, where some observations were made on the habits of the turkey vulture. Some very simple experiments with meat wrapped in paper resulted negatively, but the conditions of the experiments did not warrant the conclusion that meat is not smelled by these birds. During a tramp through a pine forest,

a turkey vulture was flushed from the entrance of a gopher-turtle hole. The bird showed a great disinclination to leave the spot although other individuals which were seen by the writer outside of cities were disposed to be wild. A dead gopher-turtle was found inside the burrow. It was impossible to view the turtle except when in a position to look down the oblique burrow, and it did not seem probable that a bird when flying overhead could see the body. A very strong odor of carrion prevailed for some distance on the lee side of the burrow.

The writer could not rule out the possibility that the vulture had found the turtle outside of the hole through its sense of vision and had later pushed the body inside, but it seemed unlikely that this had happened. The circumstances all appeared to favor the conclusion that the carrion had been smelled, even though the evidence was far from conclusive. The well known naturalist, Henry Nehrling, whose estate was the headquarters of the writer during his stay in Florida, has also conducted some experiments with turkey vultures. He placed meat under boxes, and vultures actually alighted on these covers.

On September 6, 1909, the writer attempted a simple experiment on the sense of smell in *Apteryx*, at the London Zoological Gardens. Through the kindness of Mr. Pocock, three specimens of *Apteryx mantelli* were available. These birds were kept in a pen next to one occupied by some young kangaroos. The study was made in the evening after dark at the usual feeding time for the birds.

At the advice of a keeper, earthworms were selected for the experiments, as much appreciated food. Some flower pots partly filled with soil were placed in a row at one end of the pen, and one of the pots contained the worms. A very small amount of light from a lantern was used in order to note the movements of the birds, and even the feeble light which was employed was enough to check the activity of two. The third individual approached the pots rather shyly and inserted its beak at random. On two occasions, it seemed as though contact with the worms must have been nearly or quite effected, but the bird did not discover the food at these times. Eventually, the worms were located, appar-

ently by hit or miss probing with the bill. There was no evidence of the acute sense of smell described by Benham ('06), though, of course, its existence was not disproved. The danger of serious effects upon the kangaroos from the disturbance produced by these experiments was so great that no further attempts were made by the writer.

It seems highly desirable that opportunities should be furnished at our great zoological gardens for thorough-going studies of behavior, which need not cause any injury to the animals. Little can be accomplished, as a rule, under the conditions which prevail. A few animals might be withdrawn from exhibition for a period of some weeks or months without any serious loss to the public and with much gain to science. The zoological gardens are in a position to acquire and maintain animals, which makes them the logical places for the study of species that are rare or difficult to keep in captivity.

An uncorked bottle of oil of bergamot was held within a few inches of the nostrils of two emeus, but no results were obtained.

## 8. CONCLUSIONS

The author agrees with Edinger ('08a), that a sense of smell should be expected to occur in birds. Thus on page 440, Edinger says:

That part of the brain which in man and other animals is undoubtedly concerned with the sense of smell exhibits a constant arrangement and microscopic structure, not only in them but in all vertebrates down to the cyclostomes. We are therefore justified in the conclusion that an animal which possesses this part smells even though from its behavior nothing may be safely inferred. Indeed we may judge of the importance of the sense of smell to the animal according as this organ is large or small in relation to the remainder of the brain.

Unfortunately very little is known concerning the sense of smell in the vertebrates outside of mammals, but it is highly significant in this connection, that the olfactory organs of fishes have been demonstrated to function for a sense of smell, by Parker ('10), and Sheldon ('11).

In birds with relatively large olfactory lobes, such as *Dromaeus* and *Fulmarus*, the sense of smell should of course be stronger than in the *Corvidae*. The writer knows, however, of no observations upon the sense of smell in the first two birds other than the unsatisfactory ones already mentioned in this paper.

Man is classed as a microsmatic animal, and the human olfactory organs are relatively very small; yet no one with a normal olfactory sense would deny that the human olfactory organs give their owners a large number of more or less intense impressions. It has not been practicable to make an exact comparison of the relative sizes of the organs of smell in birds and man, but those portions of the olfactory apparatus which are known in birds seem more extensive, relatively, in some birds, at least, than those of man. From a morphological standpoint, then, such birds as the Fulmar should be expected to have a more acute sense of smell than man possesses.

It is quite possible that olfactory stimuli may simply reinforce reactions to other stimuli such as visual and tactile impressions. We may have mutual relations of stimuli such as were found by Yerkes ('05 and '06), for the sense of hearing in the frog. Such birds as the duck or the flamingo, for example, may possibly, in probing for food, have tactile sensations which are received through the huge trigeminus nerves, modified by olfactory stimuli. The negative results of most experiments with vultures may have been due to a mutual relation between the olfactory and visual senses which made it difficult for the bird to react to an olfactory stimulus only.

The author agrees with Turner ('91), that the great reduction of the olfactory organs which has occurred in the higher birds would seem to indicate that the development of keen vision in birds is being accompanied by a degeneration of the olfactory sense which may result in its total loss, eventually.

Though the doves in the experiments described on pp. 646-650, never learned to find their food with perfect accuracy during a series of studies which extended, twice a day, through the greater part of about nine months, it is evident that they were stimulated by at least one of the odorous materials used, *i.e.*, oil of bergamot.

Unfortunately, the writer did not find it practicable to eliminate the possibility of stimulation of the nerves of general sensation in the experiments. The operations which would be involved in cutting all of the nerves of general sensation which might possibly be concerned were, in the writer's judgment, too severe to be worth attempting. Not only the innervation of the nasal cavities, but also that of the mouth chamber, would be involved. It has been suggested by Professor Herrick that a series of tests be made with birds whose olfactory nerves had been cut, but it has not yet been possible to attempt this desirable experiment. However, the extreme tenuity of the odorous material where stimulation occurred would appear to require a sensitivity far more acute than that which is known to be possessed by general sensory endings. A quantity of only about 5 cc. of oil of bergamot, for instance, was used and there was no significant loss in volume during the months which were occupied by the series of experiments.

In the author's judgment, the results of the ring dove experiments warrant the conclusion that the behavior of some birds at least may be affected by olfactory stimulation.

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## PLATES

Original drawings made with the aid of photographs and the Edinger projection apparatus. The cerebrum and the cerebellum have been included to indicate the relative size of the olfactory lobes.

## ABBREVIATIONS

- c. a.*, Anterior concha or turbinal.    *c. p.* Posterior concha or turbinal.  
*c. m.*, Middle concha or turbinal.    *t.*, Ophthalmic branch of trigeminus nerve.

## PLATE 1

## EXPLANATION OF FIGURES

(All figures  $\times 1$ )

1 Dorsal view of olfactory lobes and nerves in *Struthio camelus*, (ostrich). Posterior ends of nasal chambers shown semi-diagrammatically.

2 *Dromaeus novae-hollandiae*, (emeu). Dorsal view showing olfactory lobes and a portion of the nasal chambers. A posterior portion of the roof of the left nasal chamber has been dissected away to show the contour of part of the posterior turbinal. The right posterior turbinal and a posterior portion of the right middle turbinal are also exposed. Contour lines for the eyes and bill have been sketched at the sides of the drawing for orientation.

3 *Dromaeus*. Lateral view of specimen shown in fig. 2, but without the nasal chambers.

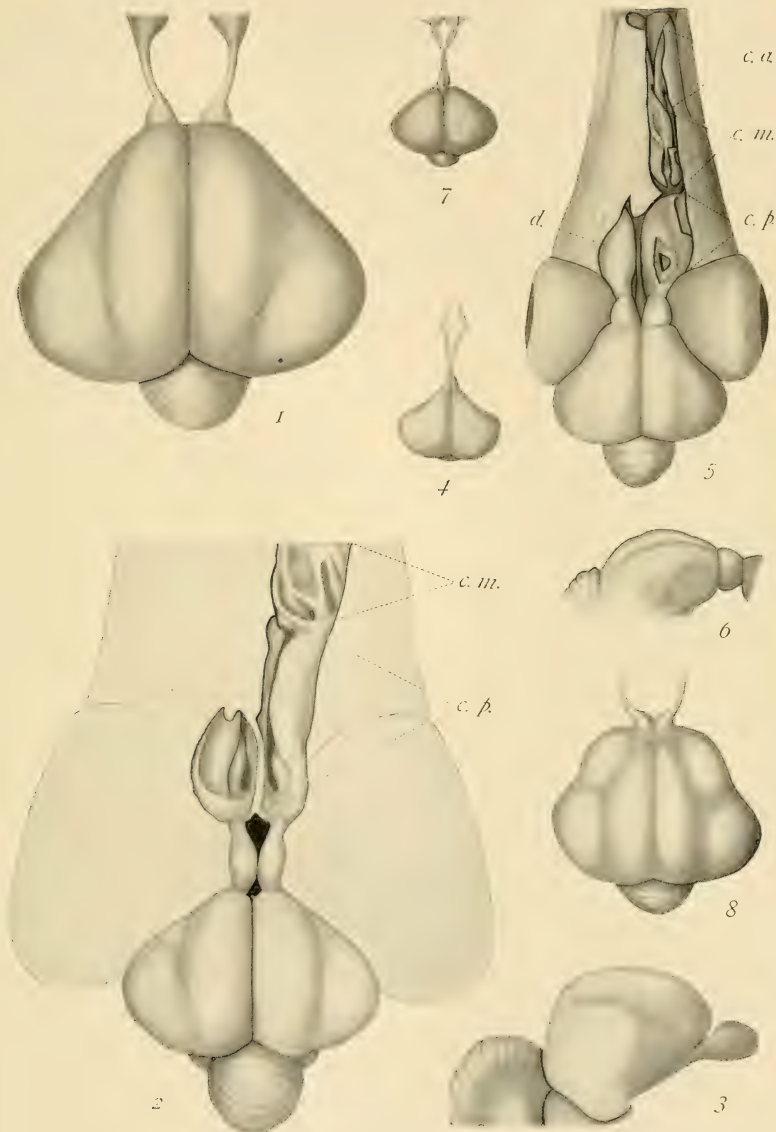
4 *Turtur risorius*, (ring dove). Dorsal view to show olfactory lobes, olfactory nerves, and sections of the ophthalmic branch of the trigeminus nerve.

5 *Fulmarus glacialis*, (fulmar). Dorsal view of portion of dissected head with the brain case material which separates the eyes from the brain removed. The right nasal chamber and a posterior portion of the left nasal chamber are exposed. The posterior turbinal of the right side has been opened at *d* to show the turns or rolls of its structure. The middle and anterior turbinals have been mutilated slightly in the dissection.

6 *Fulmarus*. Lateral view of right olfactory lobe. A small posterior bit of the right nasal chamber is represented semi-diagrammatically in contact with the right olfactory lobe.

7 *Pavoncella pugnax*, (ruff). Dorsal view. Sections of the ophthalmic branches of the trigeminus nerve are figured pulled apart very slightly to avoid confusing their outlines with those of the olfactory nerves.

8 *Pseudotantalus leucocephalus*, (white-headed ibis). Dorsal view to show olfactory lobes and nerves.



## PLATE 2

## EXPLANATION OF FIGURES

Figures 15 and 16 are  $\times 1\frac{1}{2}$ . All other figures  $\times 1$ .

9 *Leptotilus crumeniferus*, (African adjutant). Dorsal view to show olfactory lobes, olfactory nerves, and posterior portions of the nasal chambers. The ophthalmic branches of the trigeminus nerves are seen pulled slightly apart, and they enter the roof of the nasal chambers anteriorly. The main branches of the right olfactory nerve in the dorsal inner roof of the nasal chamber are shown as they appear in such a preparation. The corresponding area in the left nasal chamber has been opened.

10 *Phoenicopterus roseus*, (European flamingo). Dorsal view showing olfactory lobes, olfactory nerves, posterior portions of the nasal chambers, and ophthalmic branches of the trigeminus nerves. Contour lines for the eyes have been drawn.

11 *Catharistes urubu*, (black vulture). Dorsal view showing olfactory lobes and nerves, posterior portions of the nasal chambers, and trigeminus branches. The trigeminus nerves are seen ascending from the orbit and converging at some distance anterior to the junction of the olfactory nerves with the nasal chambers. Contour outlines for the eyes are sketched.

12 *Circaetes gallicus*, (serpent eagle). Dorsal view showing fused olfactory lobes, olfactory nerves, and posterior ends of the nasal chambers. The fused and lengthened condition of the olfactory lobes which has been shown here was not found in the other birds of prey which were examined.

13 *Chrysotis auripalliata*, (golden-naped Amazon parrot.) Dorsal view of dissected head. The olfactory lobes are seen to be fused with the diverging fore-brain lobes, and they are practically non-definable by the method of dissection alone. The olfactory nerves are seen to diverge widely. The right nasal chamber has been exposed to show the turbinals. The tissue composing the orbits has been removed, and the eyes are consequently not separated in the figure from the cerebrum and the olfactory nerves.

14 *Coccyges erythrophthalmus*, (black-billed cuckoo). Dorsal view of olfactory lobes and nerves.

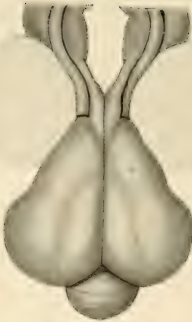
15 *Motacilla alba*, (white wagtail).  $\times \frac{3}{2}$ . Dorsal view showing the very small and fused olfactory lobes merged with the tapering fore brain lobes so as to be undefinable by the method of dissection only.

16 *Coccothraustes coccothraustes*, (hawfinch).  $\times \frac{3}{2}$ . Dorsal view of portion of dissected head. The right nasal chamber has been partly exposed. The olfactory nerves are shown throughout most of their extent. Orbit tissue removed.

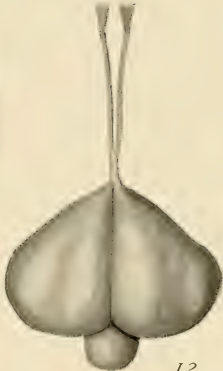
17 *Corvus corax*, (raven). Dorsal view showing the minute olfactory lobes and the slender olfactory nerves. The posterior ends of the nasal chamber are included.



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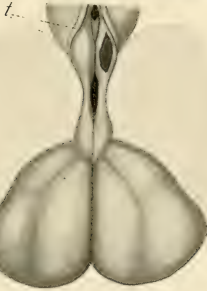
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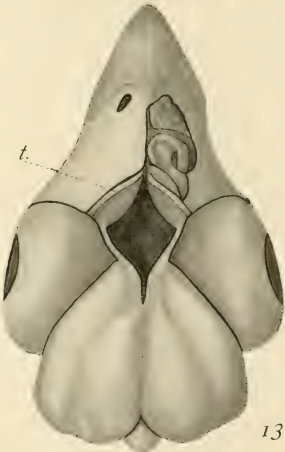
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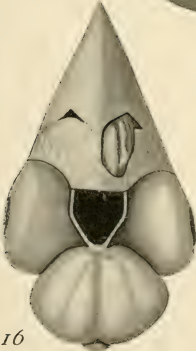
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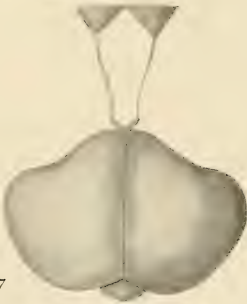
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17



# ON THE REGULAR SEASONAL CHANGES IN THE RELATIVE WEIGHT OF THE CENTRAL NERVOUS SYSTEM OF THE LEOPARD FROG

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FIVE CHARTS

The bearing of this investigation can best be understood by a short account of the steps leading up to it. In some earlier studies on the innervation of the muscles and skin of the leg of the bullfrog and of the leopard frog (Donaldson '98) (Donaldson and Schoemaker '00)<sup>1</sup> the weights of the brain and of the spinal cord of the frogs were taken, and the percentage of water in these two portions of the central nervous system determined.

When the records thus obtained were assembled, the arrangement of them as they appeared on the chart suggested that the increase in the weight of the central nervous system might run parallel to a logarithmic curve, based on the weight of the entire body. The curve based on this datum alone was however found to fall away from the observed values as the body weight increased and hence a second factor, the value of which increased gradually but at a diminishing rate, was necessary to make the calculated values correspond to those observed. This second factor was found in the total length of the frog, the fourth root of which increased at such a rate that when the logarithm of the body weight is multiplied by the fourth root of the total length, the values obtained are an almost constant fraction of those observed. It remained then merely to multiply the number thus found by a constant to approximate the observed values.

<sup>1</sup> In the paper cited above and in several other publications from my laboratory, the leopard frog has been designated *R. virescens brachycephala* Cope. Since 1908 the name *Rana pipiens* has been used (see Donaldson, *Science*, vol. 26, p. 655, 1907).

The formula for the weight of the central nervous system was accordingly written

$$\text{Weight } C. N. S. = (\text{Log. } W \times \sqrt[4]{L}) C$$

where 'weight *C. N. S.*' is the combined weight of the brain and spinal cord in milligrams; *W* the body weight in grams; *L* the total length of the frog in millimeters, and *C*, a constant empirically determined.

Corresponding results were obtained for both the bullfrog and leopard frog (Donaldson '02).

By this formula it is possible to calculate the approximate weight of the central nervous system of the frog from the data on body weight and total length, and also to show its growth.

The observations used in the foregoing study, from which the formula was obtained, were taken from summer frogs (*i.e.*, in the case of the bullfrogs, July and August, and in the case of the leopard frogs, June and July).

In commenting on these results, I pointed out at the time that it was necessary to avoid several sources of observational error. These are represented (1) By variations in the moisture of the frog, and therefore only frogs that have been kept moist for some hours at least, should be used. (2) By loss of weight during captivity, especially in frogs taken in the spring and early summer. Hence such frogs must be examined either as soon as caught or must be kept under special conditions or some correction must be made for the loss which they undergo. (3) By season; as I noted that both in the few spring and autumn frogs which I had examined, the nervous system was apparently relatively lighter than in frogs killed during the midsummer.

In the course of this work, the first two sources of error were taken into account, and corrections made where they were deemed necessary. Also, as just stated, the third was escaped by using summer frogs only.

The difference thus found between the relative weight of the central nervous system in the summer and in the spring and autumn, appeared to me worth further examination, for unless it could

be satisfactorily explained, the formula which had been suggested for determining the weight of the central nervous system seemed to have only limited applicability.

For this reason, in 1901-02, I endeavored to get data which would show whether a regular seasonal variation took place. Since that time, I have examined for the same purpose other series of frogs in 1908 and also in 1909.

The general result of these observations is to show that the relative weight of the central nervous system of the leopard frog does change during the year, being constant during hibernation, low in the spring, high in the summer and low again in the autumn, when the frogs go into hibernation.

The discussion which follows is intended to present

- I. The evidence that a seasonal change occurs.
- II. The biological interpretation of this change.

#### I. THE EVIDENCE THAT A SEASONAL CHANGE OCCURS. TECHNIQUE AND SOURCES OF ERROR

In all of the series about to be described, the technique for examination has been essentially uniform. Specimens of *Rana pipiens*, the leopard frog, alone were used. The frogs were kept moist for several hours before dissection. They were killed with chloroform and the body weight = (Bd. W.) taken to the nearest 0.1 gm. The frog was next either suspended or laid flat on its ventral surface, with the legs fully extended, and the distance from the tip of the nose to the tip of the longest toe taken with a jointed calipers and then read off on a scale to the nearest millimeter = (total length). While in the ventral position, the long axis of the head was brought in line with that of the body by raising the head with a small wooden wedge, and with a vernier calipers the distance from the tip of the nose to the tip of the urostyle—the cartilaginous end of which was exposed by a slit through the skin—was measured and read to the nearest 0.1 mm. = (body length).

The frog was then placed on its back, opened and all the viscera removed.

At this time any necessary correction in the body weight was made by subtracting from the initial weight, the weight of the ova or of undigested food distending the stomach. These were the only two corrections made to the body weight. The body weights of the females are always given without the ova.

After evisceration, the brain was exposed through its entire length and the spinal cord exposed as far down as the III nerve. With spring compasses, the length of the brain from the tip of the olfactory bulbs to a point midway between the tip of the calamus scriptorius and the level of the III nerve = (origin of II nerve) was taken. This was recorded to the nearest 0.1 mm. = (brain length) The olfactory nerves were next cut through with a very fine scissors, and in the same manner a section was made between the tip of the calamus scriptorius and the III nerve, *i.e.*, the level of the emergence of the II nerve. The choroid plexus over the fourth ventricle was removed and then the brain was raised from behind forwards on a narrow lifter, and the nerve roots severed as close to the brain as possible.

If the hypophysis was still attached to the brain, it was removed and the remaining mass at once placed in a closed weighing bottle and weighed to 0.1 milligram = (brain weight).

Similarly the spinal cord was exposed through its entire length and the conus just caudad to the XI nerve laid bare. With the spring compasses the length from this point to the level at which the cord had been severed from the brain was measured and recorded to the nearest 0.1 mm. = (cord length). The cord was then seized just below the conus with a fine forceps and raised so that the nerves could be clipped away close to the cord. The mass of the cord, thus deprived of nerves, was placed in a closed weighing bottle and at once weighed to 0.1 milligram = (cord weight.)

Both parts of the central nervous system were then dried at 90-95° C. for a week and reweighed. From these data, the percentage of water in the brain and in the spinal cord was determined to 0.1 per cent = (percentage water, brain) = (percentage of water, spinal cord).

For the present investigation, the foregoing determinations represent all that are necessary.

*Observations on the frogs examined at Chicago, 1901-1902*

Beginning in the spring of 1901, I endeavored to examine four males and four females of *R. pipiens* each week from March 28, 1901, to the following March, 1902. As will be seen from table 1, this plan met with only approximate success.

The frogs examined in Chicago were obtained from a dealer who was in turn supplied from a wide range of country extending from southern Minnesota to Indiana.

It was assumed at the beginning of this work that season was the main cause modifying the relative weight of the central nervous system in these frogs, and that therefore the fact that the frogs were taken from different localities would not materially modify the results. Consideration of all the facts at present in hand now leads me to think, on the contrary, that the relative weight of the nervous system is modified by station, and that in the case in question, the frogs from different stations were mixed together. As a rule however, the lots which were obtained were rather uniformly mixed and so, except in a few cases, no serious discrepancies appeared.

As has been stated, it was planned to examine every week, four frogs of each sex or eight in all. From March 28, 1901, to October 2, this plan was followed with moderate success, although the number of complete records is less than of those incomplete. This has come about by reason of the fact that although the full number of frogs was examined almost every week, nevertheless when the percentage of water in the brain and in the spinal cord was determined later, it was found in some cases that it was beyond the normal limits. These I have set for the brain as 83.5-85.5 per cent and for the spinal cord as 79.5-81.5 per cent. When any record transgressed these limits for *both* the brain and spinal cord, such a record was excluded as it was assumed that deviation to this extent in both divisions of the central nervous system meant that the frog was in an abnormal state.

Between October 2nd and the end of the year, this deviation in the percentage of water made it necessary to exclude all the records. The frogs obtained by us at this season had evidently

been caught much earlier and were suffering from the conditions under which they were kept.

It was then not until the end of January, 1902, that a few freshly captured frogs were brought in and these were used for series 37, the last one in table 1.

The frogs examined were selected from the dealer's tank less than 24 hours before they were to be dissected, and kept in proper

TABLE 1  
*Data on frogs from Chicago, 1901-02*

SERIES	NO. OF SPECIMENS		DATE		VALUE OF C		PERCENTAGE OF WATER	
	M.	F.			Mean	Range	Brain	Cord
1901								
1.....	3	3	March	28	24.7	(24.2-25.7)	84.1	79.5
2.....	4	1	April	3	24.8	(22.5-26.7)	84.4	80.2
3.....	4	3	April	10	25.4	(23.6-28.7)	84.7	79.8
4.....	4	4	April	16	26.9	(22.9-29.4)	84.7	80.7
5.....	4	4	April	23	27.0	(24.3-31.4)	84.2	80.2
6.....	4	4	April	30	25.0	(23.0-26.3)	85.0	80.7
7.....	4	4	May	8	28.6	(24.6-32.7)	85.1	80.9
8.....	4	2	May	15	27.3	(24.1-29.8)	84.4	80.9
9.....	3	4	May	21	28.1	(24.1-30.5)	84.9	80.5
10.....	4	4	May	28	27.2	(24.4-30.9)	84.4	80.4
11.....	3	3	June	4	29.4	(24.5-33.8)	85.0	81.0
12.....	4	3	June	11	26.1	(23.5-29.1)	84.2	80.2
13.....	2	4	June	19	26.4*	(21.5-30.1)	85.0	80.9
14.....	4	2	June	26	25.2*	(23.5-27.5)	85.5	80.3
15.....	2	3	July	3	24.2*	(22.9-25.4)	85.6	80.9
16.....	4	3	July	9	25.1*	(23.7-26.3)	84.4	79.8
17.....	4	4	July	17	27.9	(25.9-30.7)	84.3	79.7
18.....	4	3	July	23	30.0	(27.3-31.4)	84.9	80.7
19.....	4	4	July	30	29.3	(22.7-33.7)	85.0	80.5
20.....	4	4	August	6	27.0	(25.3-30.9)	84.5	79.7
21.....	4	4	August	13	29.4	(28.1-31.5)	85.1	80.4
22.....	3	4	August	20	28.0	(24.8-31.1)	84.9	81.3
23.....	4	3	August	27	28.5	(24.9-33.6)	84.6	80.4
24.....	3	3	September	3	27.1	(23.6-31.2)	84.5	79.0
25.....	2	4	September	12	29.2	(24.0-32.7)	84.1	79.1
26.....	3	2	September	17	24.9	(22.8-25.9)	85.2	80.9
27.....	3	4	September	24	28.6	(26.4-30.6)	84.3	80.2
28.....	4	4	October	2	28.1	(26.4-32.3)	84.4	80.6
1902								
37.....	2	2	January	30	24.8	(22.9-27.8)	84.6	81.1

\*See page 671.

jars in the laboratory until examined. Enough frogs were taken to give as a rule four of each sex, and the endeavor was made to have them of nearly the same size, *i.e.*, 22–29 gms. in body weight. A glance at table 2 will show that this effort was largely successful. The measurements which were made included (1) the total length, (2) the body weight (3) the weight of the brain (4) the weight of the spinal cord, as well as the length of each of these portions of the central nervous system and the percentage of water in each.

These data, from 1–4, are necessary for determining the value of the constant  $C$  in the formula

$$\text{Weight of } C. N. S. = \left( \text{Log } W \times \sqrt[4]{L} \right) C.$$

It will be seen from the inspection of the formula that if there are two frogs of the same body weight and the same total length, but differing in the weights of their central nervous systems, then in the case of the frog with the lighter nervous system, the value of  $C$  will be less than in the other, for by the terms of the formula, the weight of the central nervous system is

$$C \text{ times } \left( \text{Log } W \times \sqrt[4]{L} \right)$$

a value which in this instance is the same for both the frogs. It becomes therefore possible to express the changes in the relative weight of the central nervous system by the differences in the value of  $C$ —and it is this method which has been here employed.

This variation in the constant  $C$  not only enables us to express the variations in the relative weight of the central nervous system, but is by far the best method available for the purpose, as it is quite independent of the absolute size of the frog in any instance.

In table 1, the average value of  $C$  is given together with the range in this value for each series.

If these average values for successive weeks are plotted, they show great irregularity. By grouping the series however, so as to take the first one alone, then the next three series as the second group, and finally the remaining series up to the very last one in groups of four, and the last one again alone, we obtained the data

TABLE 2

*Data on frogs from Chicago, 1901-02. Fundamental table giving the mean values of all the data for each group*

SERIES	NUMBER OF SPECIMENS		BODY WEIGHT	TOTAL LENGTH
	M.	F.		
			<i>gms.</i>	<i>mm.</i>
1.....	3	3	21.2	165
2-4.....	12	8	22.6	170
5-8.....	16	14	21.3	168
9-12.....	14	14	22.3	168
13-16.....	12	12	24.4	174
17-20.....	16	15	22.4	166
21-24.....	14	14	23.3	166
25-28.....	12	14	23.0	165
37.....	2	2	19.4	166

*Brain*

SERIES	WEIGHT	LENGTH	PERCENTAGE OF WATER
	<i>gms.</i>	<i>mm.</i>	
1.....	0.0793	13.7	84.1
2-4.....	0.0857	13.7	84.6
5-8.....	0.0870	13.6	84.7
9-12.....	0.0917	13.9	84.6
13-16.....	0.0859	14.1	85.1
17-20.....	0.0939	14.2	84.7
21-24.....	0.0944	14.2	84.8
25-28.....	0.0933	14.1	84.5
37.....	0.0793	13.4	84.6

*Cord*

1.....	0.0382	14.6	79.5
2-4.....	0.0410	15.6	80.3
5-8.....	0.0415	15.5	80.7
9-12.....	0.0423	15.3	80.6
13-16.....	0.0416	15.6	80.5
17-20.....	0.0443	15.5	80.1
21-24.....	0.0453	15.3	80.4
25-28.....	0.0429	15.2	80.2
37.....	0.0344	15.4	81.1

given in table 3. This table shows that the mean value of  $C$  rises from March 28th to a maximum in July and then begins to fall. In the case of one group, formed by the series 13, 14, 15 and 16, there appears an unexpectedly small relative weight of the central nervous system, while at the same time there is no ground to exclude these series as abnormal. I assume therefore that the series forming this group came from localities where the frogs had a proportionately small nervous system and that these were not mixed as in the case of the other series, with frogs from more northern stations, in which the nervous system happened to be larger.

For reasons given earlier, the autumn fall is very incompletely shown, but the observation of the January series gives, as one would expect, a value of  $C$  similar to that found in March. As a control, I have calculated the probable error of the mean ( $\pm .6745 \frac{\sigma}{\sqrt{n}}$ )

of  $C$  in all the groups of table 3.

As will be seen, in the groups that consist of four series, this is quite constantly about 0.3. This value is high but when it is considered that the ranges of the value of  $C$  in the several series

TABLE 3  
*Data on frogs from Chicago, 1901-02*

SERIES	NUMBER OF SPECIMENS		MEAN DATE		VALUE OF <i>C</i>		PERCENTAGE OF WATER	
	M.	F.			Mean	Probable error of mean	Brain	Cord
			<i>1901</i>					
1.....	3	3	March	28	24.7	±0.16	84.1	79.5
2-4.....	12	8	April	9	25.8	±0.32	84.6	80.3
5-8.....	16	14	May	4	27.0	±0.29	84.7	80.7
9-12.....	14	14	June	1	27.6	±0.31	84.6	80.6
13-16.....	12	12	June	29*	25.8	±0.27	85.1	80.5
17-20.....	16	15	July	27	28.5	±0.31	84.7	80.1
21-24.....	14	14	August	24	28.3	±0.30	84.8	80.4
25-28.....	12	14	September	22	27.9	±0.32	84.5	80.2
			<i>1902</i>					
37.....	2	2	January	30	24.8	±0.67	84.6	81.1

\*See Comments on p. 671.

are much the same (table 1) then it would follow that the size of the probable error of the mean would rapidly reduce as the number of cases was increased. It is by reason of this fact that I still consider the successive mean values of  $C$  significant, despite the large probable error.

When the data in table 3 are put in the form of a chart (1) where the ordinates represent the values of  $C$  on a base line of time in days, the relations above described are shown clearly. We conclude therefore from this series that the relative weight of the central nervous system of the leopard frog rises from the time that the frog appears in the spring until midsummer, and

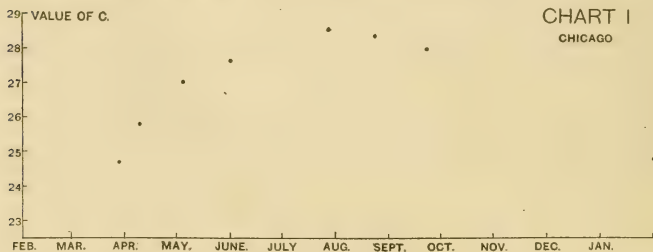


Chart 1 Based on data in table 3. Determination for  $C$ , June 29, not entered.

then falls until the frog goes into hibernation, *i.e.*, when the external temperature drops to  $7^{\circ}$ – $10^{\circ}$  C. =  $45^{\circ}$ – $50^{\circ}$  F. (Torelle, '03). It should be added however that this critical temperature is probably modified by latitude and tends to become lower as we pass from south to north in the range of the frog.

In table 2 are given the necessary fundamental data relating to the several groups belonging to this lot of Chicago frogs.

#### *Observations on the frogs from Minnesota, 1908*

The exact locality of the frogs in this series is not known. They came however from Minnesota, probably from southern Minnesota and all from the same station. They were delivered in good condition in Philadelphia where they were examined. As

table 4 shows, there were but three series and the numbers, except in the last series are small. The treatment of these Minnesota frogs was similar to that for the Chicago frogs, and need not be again described.

As shown in table 5, there is a spring series, a summer series and a late autumn series (entered in two parts) and in these three series the relative weight of the central nervous system as shown by the values of  $C$ , undergoes a seasonal variation corresponding to that found in the Chicago frogs—although the absolute values for  $C$  are much higher. This variation is exhibited in chart 2.

TABLE 4

*Data on frogs from Minnesota, 1908. Fundamental table giving the mean values for each series*

SERIES	NUMBER OF SPECIMENS		BODY WEIGHT	TOTAL LENGTH	BODY LENGTH
	M.	F.			
			<i>gms.</i>	<i>mm.</i>	<i>mm.</i>
1.....	2	2	50.5	212	79.5
2.....	0	5	56.4	225	84.2
3.....	3	3	48.5	213	78.4
3'.....	3	9	64.9	230	85.4

#### *Brain*

SERIES	WEIGHT	LENGTH	PERCENTAGE OF WATER
	<i>gms.</i>	<i>mm.</i>	
1.....	0.1250	16.4	84.8
2.....	0.1475	17.5	85.6
3.....	0.1245	16.6	85.0
3'.....	0.1334	17.5	84.6

#### *Cord*

1.....	0.0577	18.5	79.9
2.....	0.0640	18.7	80.4
3.....	0.0582	18.1	80.1
3'.....	0.0665	19.3	79.9

For the Chicago frogs we had no October observation, but in this case we do have one and it is seen that it occupies the position which we should expect, and thus supplements and extends the Chicago data.

When first computing the values of  $C$  for the Minnesota frogs, taken in October, it seemed important to use specimens having the same body weight as those used in the preceding series, so the first entry for October made in tables 4 and 5 is for the six specimens having an average body weight of 48.5 gms. This entry is designated 3. Later the value of  $C$  was determined for the re-

TABLE 5  
*Data on frogs from Minnesota, 1908*

SERIES	NUMBER OF SPECIMENS		DATE		VALUE OF <i>C</i>		
	M.	F.			Mean	Probable error of the mean†	Range
1908							
1.....	2	2	March	26	28.1	±0.30	(27.0-29.4)
2.....	0	5	June	10	31.1	±0.65	(28.8-34.8)
*3.....	3	3	October	19	28.2	±0.66	(24.5-32.6)
†3'.....	3	9	October	19	28.3	±0.36	(25.1-32.1)

\* Series 3 (six cases) has an average body weight of 48.5 gms.

† Series 3' (twelve cases) has an average body weight of 64.9 gms.

‡ See comments on pp. 671-672.

maintaining twelve specimens having an average body weight of 64.9 gms. This latter record is entered in the tables as 3'. The value of  $C$  is the same in both series. This gives me the opportunity to correct a statement previously made, (Donaldson '10, p. 14) to the effect that the value of  $C$  is in a measure influenced by the absolute size of the frog. This conclusion I now think erroneous.

It may be added that in the paper just cited the argument is not altered by the introduction of the data for the groups of frogs there excluded from comparison on account of their body weight. The most striking difference between the observations on the Minnesota frogs and those on the Chicago frogs is the high value

of  $C$  in the former, but the discussion of this point will be deferred until the observations on the next lot of frogs have been presented.

The probable error of the mean values of  $C$  given in table 5 is open to the same interpretation as was given in the case of the Chicago frogs (see pp. 671-672).

We conclude from this study of the Minnesota frogs that the relative weight of the central nervous system is low in the spring, high in the midsummer and low again in the autumn, and these relations are shown on chart 2.

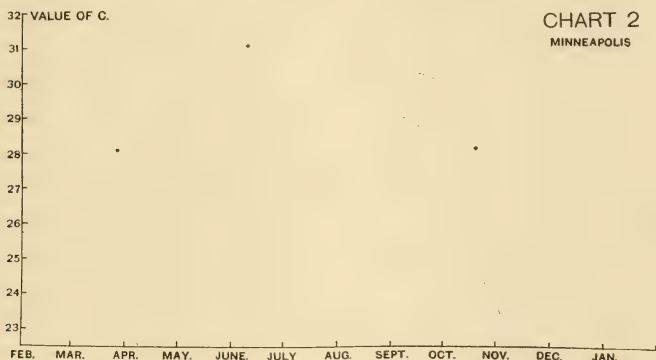


Chart 2 Based on data in table 5.

### *Observations on the frogs from the Brandywine, 1909*

These frogs were brought from the Brandywine Creek near Philadelphia, and in some ways represent the best of the three lots. The plan was to examine about twelve specimens at intervals of a month or less between the appearance of the frogs in the spring and their disappearance in the autumn. The two February series, as well as those of October and November, were taken within a spring house, and those of the intervening series from the neighborhood. The frogs did not emerge until the end of March. The July series was overheated in transport and could not be used. In all there are eleven series recorded.

As in the other cases, all the frogs examined in which the percentage of water was normal, have been included in the records, but those with abnormal percentages of water in the central nervous system have been omitted, hence many of the series contain less than twelve records (see table 6), which also gives the fundamental data for this lot.

TABLE 6

*Data on frogs from the Brandywine, 1909. Fundamental table giving the mean values for each series*

SERIES	NUMBER OF SPECIMENS		BODY WEIGHT	TOTAL LENGTH : BODY LENGTH	
	M.	F.			
			<i>gms.</i>	<i>mm.</i>	<i>mm.</i>
1.....	5	5	30.3	176	66.8
2.....	6	5	29.3	172	66.2
3.....	7	4	26.7	166	63.2
4.....	12	0	21.0	158	60.1
5.....	5	6	32.7	188	71.7
6.....	6	2	27.7	179	63.8
7.....					
8.....	3	5	28.8	176	66.3
9.....	5	7	45.5	197	75.8
10.....	2	6	40.5	190	71.5
11.....	4	7	32.1	176	67.0
12.....	2	3	33.0	182	68.9

*Brain*

SERIES	WEIGHT	LENGTH	PERCENTAGE OF WATER
	<i>gms.</i>	<i>mm.</i>	
1.....	0.0881	14.7	83.9
2.....	0.0876	14.5	83.6
3.....	0.0867	14.6	84.2
4.....	0.0729	14.4	85.0
5.....	0.1074	15.4	85.3
6.....	0.0895	14.2	84.6
7.....			
8.....	0.0962	15.0	85.0
9.....	0.1178	15.9	84.8
10.....	0.1058	15.3	85.2
11.....	0.0915	14.7	84.8
12.....	0.0927	15.0	84.8

*Cord*

SERIES	WEIGHT	LENGTH	PERCENTAGE OF WATER
1.....	0.0394	16.1	79.9
2.....	0.0382	16.4	78.9
3.....	0.0354	14.8	79.4
4.....	0.0334	15.0	80.3
5.....	0.0429	16.5	80.4
6.....	0.0361	15.8	79.1
7.....			
8.....	0.0417	15.7	80.9
9.....	0.0497	17.6	80.0
10.....	0.0446	17.2	80.4
11.....	0.0392	16.3	80.7
12.....	0.0404	16.6	80.6

When the values of  $C$  for these frogs are plotted as in chart 3, we obtain a series of records which, although irregular for the midsummer season, fit very fairly with the entries in the two preceding charts. As in the Chicago lot the average value of the probable error (table 7) is 0.3. The size of the error in this case is open to the same explanation as was given before in the cases of the Chicago and Minnesota lots. The diminution on the relative weight of the central nervous system in the autumn is shown better in this series than in either of the two preceding.

The data as they stand justify in this case the conclusion which has been drawn from the other two sets of data; namely, that the relative weight of the central nervous system is low in the spring, high in the midsummer and low again the autumn. It will be noticed that the absolute values for  $C$  are again different from those in the preceding cases, being the lowest of all.

It is hard to compare the ranges of  $C$  on the charts 1, 2 and 3 because of the difficulty of constructing satisfactory curves by which to make the comparison. I made for my own use however a series of curves, employing each to control and correct the others and obtained the following relations of  $C$ , always taking the late March value as the initial one.

The Chicago frogs were found to range in the value of  $C$  from 24.7–28.5 or 3.8 points, the Minnesota frogs from 28.1–31.3 or 3.2 points, and the Brandywine frogs from 24.0 to 27.0 or 3.0

points. Thus the percentage gains are 15.4 per cent, 11.4 per cent and 12.4 per cent respectively. This indicates that the frogs from the several localities change not only in the same manner, but also to about the same extent.

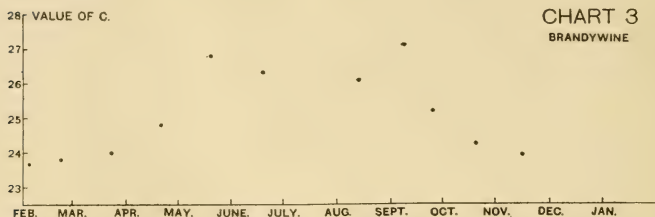


Chart 3 Based on data in table 7.

When we take the mean of the percentages given above, we find it to be 13.1 per cent, the true maximum in all cases falling in July. This result can be controlled by another treatment of the data.

TABLE 7

*Data on frogs from the Brandywine, 1909*

SERIES	NUMBER OF SPECIMENS		DATE	VALUE OF <i>C</i>		
	M.	F.		Mean	Probable error of mean*	Range
1909						
1.....	5	5	February 4	23.7	±0.20	(21.9-25.1)
2.....	6	5	February 22	23.8	±0.36	(21.7-26.8)
3.....	7	4	March 23	24.0	±0.29	(20.9-26.0)
4.....	12	0	April 21	24.8	±0.20	(23.2-26.3)
5.....	5	6	May 20	26.8	±0.61	(22.5-31.7)
6.....	6	2	June 19	26.3	±0.30	(24.4-28.9)
7.....						
8.....	3	5	August 13	26.1	±0.24	(24.1-27.1)
9.....	5	7	September 8	27.2	±0.31	(25.2-30.6)
10.....	2	6	September 24	25.2	±0.57	(21.6-29.6)
11.....	4	7	October 19	24.5	±0.43	(20.2-28.7)
12.....	2	3	November 16	23.9	±0.16	(22.7-26.0)

\*See comments on pp. 671-672.

TABLE 8

*The percentage changes in the value of  $C$  in each of the three lots of frogs. The March value used as the standard is underlined*

CHICAGO LOT				MINNESOTA LOT				BRANDYWINE LOT			
DATE		VALUE OF $C$	PERCENTAGE CHANGE	DATE		VALUE OF $C$	PERCENTAGE CHANGE	DATE		VALUE OF $C$	PERCENTAGE CHANGE
March	28	<u>24.7</u>	0	March	26	<u>28.1</u>	0	February	4	23.7	-1.0
April	9	25.8	4.8					February	22	23.8	-0.8
May	4	27.0	9.3					March	23	<u>24.0</u>	0
June	1	27.6	11.7	June	10	31.1	10.7	April	21	24.8	3.3
June	29							May	20	26.8	11.6
July	27	28.5	15.4					June	19	26.3	9.6
August	24	28.3	14.6					August	13	26.1	8.7
September	22	27.9	13.0					September	8	27.2	13.3
				October	29	28.2	0.3	September	24	25.2	5.0
								October	19	24.5	2.1
January	30	24.8	0.4					November	16	23.9	-0.4

The percentage change in the value of  $C$  as indicated by the observations when the late March value is taken as the standard, is given for all three lots in table 8.

When the data on these tables are put in the form of a chart (chart 4) and then an ideal symmetrical curve is drawn a number of interesting relations come into view.

In the first place the maximum of this curve in July is 13 per cent above the initial value of  $C$ . Second, during the month from the end of March to the end of April,  $C$  increases about 7 per cent; during the month from the end of April to the end of May, about 4 per cent, and from the end of May to the end of June, about 2 per cent. During July little change occurs and then the converse changes follow during the three months from August first to the end of October. During hibernation, November first to the end of March, the value of  $C$  is nearly constant.

These values are admittedly only approximations, but when so understood, they serve to show the general course of the seasonal changes.

From the foregoing we are justified in concluding that there is a seasonal change in the relative weight of the central nervous system of the leopard frog, *R. pipiens*, and that this occurs regularly each year and in frogs taken from widely separated localities.

Moreover, if we know in any case the value of  $C$  for a colony of frogs at a given date, it is possible in accordance with these results to determine approximately what the value will be for other representatives of the same colony, at any other season of the year.

Nevertheless in the first instance, the values of  $C$  for a given colony must always be determined by direct observation. We have seen that at similar dates the value of  $C$  for the Brandy-

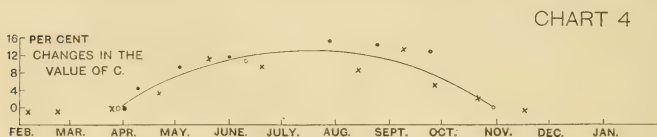


Chart 4 Based on data in table 8. Also giving an ideal curve about which the several records are grouped.

- Records from Chicago series.
- ° Records from Minneapolis series.
- × Records from Brandywine series.

wine frogs is 24.0, for the Chicago frogs 24.7 and for the Minnesota frogs 28.1. The differences between these series I refer to the general effect of the external conditions (= food supply, abundance of water, etc.) but whatever the explanation is, such differences must always be anticipated.

Further, the individual variation in this character is large so that all determinations should be based on data from groups, and not on single cases.

On the other hand, although the frogs from a given locality or station may have the central nervous system developed in a proportion different from that found in frogs from another locality, yet frogs from the same locality tend to remain constant in this character.

This last statement is supported by my observations on the two European species, *R. esculenta* and *R. temporaria*, as given in table 9.

As is seen, the value of *C* in both the European forms is less than in the American *R. pipiens* (Donaldson, '08 and '10).

The point to be specially illustrated in table 9 is, however, that frogs from the same locality maintain the same values of *C*. Thus both series of *R. esculenta*, taken from the same station at Zürich and examined in July, but with an interval of five years, show values of *C* nearly alike.

TABLE 9

SPECIES	NUMBER OF SPECIMENS	EXAMINED	VALUE OF <i>C</i>	
			Mean	Probable error of the mean
<i>R. esculenta</i> .....	11	July 20, '04	23.8	±0.46
<i>R. esculenta</i> .....	11	July 6, '09	23.7	±0.36
<i>R. temporaria</i> .....	12	July 1, '04	22.8	±0.47
<i>R. temporaria</i> .....	16	August 20, '09	21.8	±0.26

On the other hand, the two series of *R. temporaria* from the same station at Liverpool, give on July 1, 1904, 22.8 and on August 20, 1909, 21.9; a fall of about 4 per cent in the value of *C*, which, as explained above, is the sort of change to be expected, although the amount of it is larger than we should have predicted.

## II. THE BIOLOGICAL INTERPRETATION OF THE SEASONAL CHANGE IN THE RELATIVE WEIGHT OF THE CENTRAL NERVOUS SYSTEM

In order to form a proper picture of the manner in which this change in the relative weight of the central nervous system as just described is brought about, it will be necessary to obtain a notion of the growth changes in the entire frog during the active period of each year. At present only two sets of observed facts are available: (1) The change in the percentage of water. (2) Changes in length:—but from these latter, changes in body weight can be fairly inferred.

1. *The changes in the percentage of water*

As to the percentage of water in the entire frog, I made the determination in the Chicago frogs by drying the animals for several weeks in the same oven which was used for the determination of the percentage of water in the brain and spinal cord.

From each series in the Chicago lot, with the exception of 1, 2, 3 and 38, one male and one female frog were taken; both were weighed fresh and then opened and the ova removed from the female when necessary. Care was taken of course that no other tissue was lost by the operation.

The results are given in table 10, first for single series, then for the averages of the several series grouped in the way in which the series appear in table 3. The entries for the series, 13, 14, 15 and 16, which series were not used for the determination of *C*, are also given, and it is interesting to note that there was nothing peculiar in the amount of water in the entire frog in these cases.

TABLE 10

*On the amount of water in the entire frog. Chicago frogs, by single series and by groups*

PERCENTAGE OF WATER								
SERIES	DATE		MALES		BODY WEIGHT	FEMALES		BODY WEIGHT
			SERIES	GROUP		SERIES	GROUP	
	1901				gms.			gms.
1.....	March	29						
2.....	April	4						
3.....	April	10						
4.....	April	17	78.7	78.7	21.6	81.4	81.4	16.6
5.....	April	24	80.1	80.5	22.9	81.0	80.7	21.3
6.....	May	1	79.7			80.8		
7.....	May	8	80.1			80.8		
8.....	May	15	82.1			79.3		
9.....	May	22	81.1	80.4	22.7	81.4	81.2	23.4
10.....	May	29	81.4			82.2		
11.....	June	5	80.3			81.8		
12.....	June	12	78.7			79.4		

TABLE 10—CONTINUED

SERIES	DATE		PERCENTAGE OF WATER					
			MALES		BODY WEIGHT	FEMALES		BODY WEIGHT
			SERIES	GROUP		SERIES	GROUP	
	1901				<i>gms.</i>			<i>gms.</i>
13.....	June	19	80.5	80.1	22.9	82.8	80.3	23.8
14.....	June	26	79.9			79.9		
15.....	July	4	79.8			79.2		
16.....	July	11	78.4			79.4		
17.....	July	18	78.9	78.7	22.9	79.7	78.4	21.7
18.....	July	24	80.4			78.1		
19.....	July	31	77.9			76.8		
20.....	August	7	77.8			78.9		
21.....	August	14	76.5	77.4	24.4	77.6	76.9	22.4
22.....	August	21	76.5			76.5		
23.....	August	28	78.1			77.3		
24.....	September	4	78.7			76.3		
25.....	September	12	76.3	78.2	24.4	75.2	77.3	24.0
26.....	September	18	81.3			78.4		
27.....	September	25	76.8			77.5		
28.....	October	3	78.5			78.2		
29.....	October	10	78.4	79.0	24.2	78.6	79.9	22.4
30.....	October	17	77.8			81.9		
31.....	October	24	79.4			78.5		
32.....	October	31	80.5			80.7		
33.....	November	14	79.8	80.7	23.3	80.2	80.7	24.3
34.....	November	27	81.3			82.0		
35.....	December	13	81.0			80.0		
	1902							
36.....	January	10	82.3	82.3	16.6	84.5	83.2	16.3
37.....	January	31	82.3			81.9		
38.....	March	22						

The entry for January 10 and 31, 1902, of M. 82.3 and F. 83.2 per cent seems very high—but the water in the nervous system of these groups was not found to be excessive. These high values are probably due to the fact, as suggested by the body weights, that these frogs were a year younger than those used for the rest of the series. I will return to this matter later. On following

down the water determinations by groups, it is quite evident that the entire frog begins with a high percentage of water in April and May, which diminishes towards the midsummer (August) and then rises again during the autumn. Moreover as the records stand, they suggest that the percentage of water in the female, as compared with the male, is higher in the spring, lower from July to October and higher again during hibernation.

It may be added as bearing on this difference according to sex that I made a few observations on the percentage of water in the ripe ova of these frogs. These were taken early in the spring. The determinations are given in table 11.

TABLE 11  
*Percentage of water. Females*

	IN ENTIRE FROG WITH OVA	IN SAME FROG WITHOUT OVA	IN OVA
Series 4.....	76.8	81.4	56.4
Series 5.....	76.8	81.0	63.2
Series 7.....	76.5	80.8	52.3

The average of the values in table 11 is 57.3 per cent which is in general agreement with the old observations of Beaudimont and St. Ange ('47) giving in the eggs of *Rana (esculenta?)* the percentage of 55.7.

The data serve to show the relatively small amount of water in the ova and the effect of the presence of the ova in reducing the percentage of water in the entire frog. It is just possible that in late summer, at least, small quantities of young ova, considered at the time too insignificant to be removed, may have contributed to the lower percentage of water in the female at this season.

For the general course of this percentage during the season, as shown in table 10, it is difficult to give a complete explanation. Long ago, in his admirable study on the distribution of water, v. Bezold ('57) showed that larger frogs (*Rana temporaria*) had a less percentage of water than smaller ones. His series ranged in body weight was from 3.0-61.0 gms. and the corresponding percentages of water were 79.77 and 74.31, with six intermediate determinations.

In this series it is to be noted that the heaviest frogs are probably three years older than the lightest. At 22.7 gms., v. Bezold finds the percentage of water in the early summer to be 78.2, which is in good agreement with my July record of 78.7 (average of both sexes) for frogs the average body weight of which was 22.4 gms.

Since in table 10 the body weight values—save for April (females) and January (both sexes)—are nearly alike, the variations in the percentage of water cannot well be explained as due to size. On the other hand if the frogs were from eggs of the same year, except in the instances above indicated, the specimens examined later in the season must be older than those taken earlier. Advancing age would demand a fall in the percentage of water and this fall as we have seen, occurs. But the fall in turn gives way to a rise in the percentage of water after the beginning of September. To explain this latter result, I can merely suggest that it occurs as active feeding comes to an end and when the frog is less well supplied with food than in the early part of the summer, and thus it may represent a condition of underfeeding or starvation which has been shown by Moraczewski ('00) to cause the percentage of water in the entire frog to rise.<sup>2</sup>

It is evident therefore that age and food conditions at least have an influence on the percentage of water in the entire frog, but the results, like those given in table 10, cannot be fully explained until it is determined first whether there are additional important conditions, and second, how these conditions which we do recognize interact.

Before leaving this topic it is important to state that in both the brain and the spinal cord no systematic variation in the percentage of water can be observed during the active season. This statement is true for all three lots of frogs. Moreover, as shown in table 12, the averages for the percentages of water in the brain and cord (using the data in tables 3, 4 and 6) are nearly alike for all three lots of frogs.

<sup>2</sup> In the paper cited above, Moraczewski's general conclusion 2, page 144, is contradictory to his tables. The above statement in the text is based on the tables and on the text on page 136 of the paper cited.

TABLE 12

*Average values of the percentages of water in the brain and spinal cord of the several lots of frogs, from data in tables 1, 3 and 6*

	BRAIN	CORD
Frogs from Chicago.....	84.6	80.4
Frogs from Minnesota.....	85.0	80.1
Frogs from the Brandywine.....	84.6	80.0

## 2. Changes in length

On the growth of the frog in length during the active season only two sets of data have been found. In the first instance Miss Dickerson ('07) gives pictures of *Rana aurora* at one, two and three years. On measuring these, I obtained the body lengths given in table 13.

Using the determinations made on *R. pipiens*—which *Rana aurora* resembles—and according to which the body length is 37.5 per cent of the total length—we obtain the calculated total lengths given in table 13.

From a series of determinations of the relation of body weight to total length in *R. pipiens*, Dr. Hatai ('11) has developed the accompanying formula:

$$y = 158 \text{ Log}(x + 6.5) - 63$$

in which  $y$  is the total length in millimeters and  $x$  the body weight in grams. This expresses the normal relation of total length to body weight in *R. pipiens*<sup>3</sup>.

TABLE 13  
*Rana aurora*

AGE	BODY LENGTH	CALCULATED	
		Total length	Body weights
<i>yrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>gms.</i>
1.....	36	96	3.6
2.....	50	133	10.9
3.....	63	168	22.5

<sup>3</sup> This formula was based on the measurements of several series of Chicago frogs, *R. pipiens*. It fits the observations on the Minnesota frogs also. The observa-

When this formula is applied to the foregoing data, we obtain for the given total lengths the body weights which are entered in table 13. The results show that for the years taken, the body weight approximately doubles during each active season. This completes the first instance.

The second instance is from Fischer-Sigwart ('97) who reports for *R. temporaria* the following body lengths at different ages: see 'body length' in table 14.

TABLE 14  
*R. temporaria*

AGES	BODY LENGTH	CALCULATED	
		Total length	Body weight
	<i>mm.</i>		<i>gms.</i>
End of first year.....	20-25	68	0.8
End of second year.....	30-35	95	3.5
End of third year.....	(42-47)*	(128)*	8.0*
End of fourth year.....	55-60	163	22.0

\*Interpolated by H. H. D.

According to Boycott ('04) the body length in *R. temporaria* is 36.6 per cent of the total length.

If now we take for the determination of the total lengths the highest values for the body lengths as given in the foregoing table, we obtain the series of figures marked 'total length' in table 14. Using the data on body weight given for *R. temporaria* by Boycott ('04) in his table (p. 375) we obtain the approximations for the body weights which are given in the last column of table 14.

Here again the body weights are more than doubled from season to season during the last three years. The value of the foregoing calculations lies not in the exact numbers obtained, for these are in a measure open to correction, but in the indication which these numbers give of the rate of growth from year to year.

tions on the Brandywine frogs however show for a given body weight, total lengths about 4 per cent less than those determined by the formula. These last frogs are therefore heavier for a given total length or shorter for a given body weight than those on which the formula is based. The formula does not apply to frogs less than 3.5 gms. in body weight.

They show that the rate is such as to cause the body weight to double, or more than double, from season to season during the three successive annual intervals; a very peculiar interesting result when compared with the growth of mammals.

Having thus determined the growth in body weight from season to season, it is desirable to calculate the weights of the central nervous system which correspond to the body weights found. We shall take but one instance—namely the last pair from the table 14—as these represent records for which we have some control observations.

The weight of the central nervous system is determined by the formula based on body weight and body length, using in the first instance 20.2 for the value of  $C$ . This value of  $C$  was obtained in the following manner. On referring to table 9, page 681, it is seen that the value of  $C$  for *R. temporaria* on July 1, 1904, was 22.8. According to our present view of seasonal change, we should expect this to be the maximum annual value of  $C$ . If this be correct, then this value is 13 per cent greater than it would be in the spring or autumn; therefore at the two ends of the season we should expect the value of  $C$  to be 13 per cent less or 20.2. The weight of the central nervous system is therefore calculated accordingly, *i.e.*, with  $C = 20.2$  for the two ends of the season.

In addition to the two sets of values giving the body weight, total length and weight of central nervous system, first at the time of emergence of a given individual, and second at the time of its hibernation, there have been interpolated in table 15 the values for this same frog when half grown in body weight—that is weighing 15.0 gms., and in the first instance the weight of the central nervous system in this half grown frog is calculated using 20.2 as the value of  $C$ .

This table 15 gives us a notion of what would take place if the frog increased by about two and a half times its initial weight in the course of the season, and at the same time underwent the normal correlated increase in body length and in the weight of its central nervous system—the relative weight of this latter remaining the same, *i.e.*, the constant  $C$  remaining unaltered during the process and having the value of 20.2. From the Zürich series we have reason to think, (see table 9,) that the mid season value of

$C$  is not 20.2 but 22.8, as observed. When this latter value of  $C$  is taken, then the weight of the central nervous system at the mid season becomes 0.0941 gms., which is nearly the weight found at the end of the season. This value is entered in table 15 under  $C = 22.8$ .

The first comment on these results is that they are in good agreement with the direct observations on the weight of the central nervous system in *R. temporaria* (Donaldson, '08, table 9), and may therefore be used as a basis for further argument.

The foregoing computations have been repeated in the case of the data for *R. aurora*, (table 13), but as the results depend entirely on the fact that the frog more than doubles its body weight

TABLE 15  
*R. temporaria*

TOTAL LENGTH	BODY WEIGHT	CALCULATED WEIGHT OF CENTRAL NERVOUS SYSTEM	
		$C=20.2$	$C=22.8$
<i>mm.</i>	<i>gms</i>	<i>gms</i>	<i>gms</i>
128	8.0	0.0614	
150	15.0	0.0834	0.0941
163	22.0	0.0968	

from season to season, and as we do not have data to control them, it does not appear necessary to put down the numerical findings.

If now we attempt to picture how these growth changes which have been determined are related to one another in order to give the results found, the following appears.

As shown in table 15, the weight of the central nervous system at emergence is .0614 gms. and at hibernation .0968 gms., a gain of 57 per cent. For the mid weight value in this table, or a body weight of 15.0 gms., the weight of the central nervous system would be .0834 if  $C$  remained constant. We know however that  $C$  rises in the first half of the season and in July is 13 per cent greater than in March. Taking  $C$  as 22.8 therefore, or 13 per cent more than its initial value, the weight of the central nervous system becomes .0941, or almost that found at the end of the

season. We conclude from this that the growth of the central nervous system is precocious and takes place mainly in the first half of the active season.

The relations just described are plotted in chart 5. Here the shape of the curve for the increasing weight of the central nervous system is fixed for the last half of the season, but the form given to it for the first half is based on the assumption that growth must begin slowly and become rapid only later.

Until direct observations on the body growth can be made for the exact control of this curve, the form given here may stand as a probable representation of what occurs.

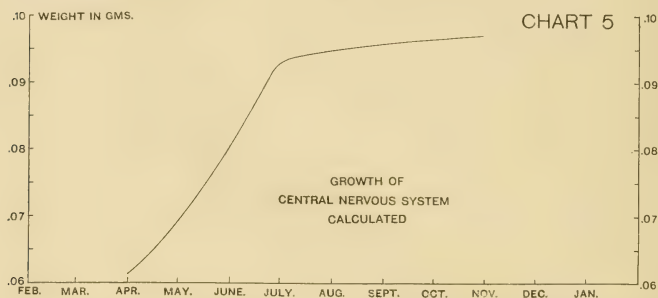


Chart 5 Curve for the growth of the central nervous system, *R. temporaria*. Based on data in table 15, using the value of  $C=20.2$  for March and October and the value of  $C=22.8$  for July.

It is to be remembered however that this curve as it stands is based on observations on *R. temporaria*, but from what we know about this species it seems most probable that it applies to *R. pipiens* also.

In connection with the phenomena just described, it may be well to review briefly the growth conditions for the frog at various seasons. When a normal frog disappears in hibernation, it is prepared for the experience. The digestive system has suffered involution and a considerable amount of fat has been stored in the fat bodies, liver and muscles. The frog emerges from hibernation with most of this stored material intact and lives on it largely during the breeding season and the earlier spring weeks.

As this gradually becomes exhausted, feeding is resumed and with the advance of the season and the increase in food, the frog not only grows, but restores these reserves and prepares for the next period of hibernation.

This review of the feeding habits of the frog serves to emphasize the fact that the conditions for nutrition in the early part of the season are different from those to be found later, and in so far might be responsible for the peculiarities in the relative growth of the central nervous system which we have observed.

In addition there are several considerations which have a very immediate bearing on the foregoing results, and especially on their variability. It must always be remembered that we are working with an animal in which the regulation of both general and relative growth is poor: an animal very responsive to the influence of external conditions—one that can be chilled or warmed, dried or made moist, fed abundantly or left without food for long periods.

Thus, in a poor season, *i.e.*, poor in insects, or in the water conditions, the frog may not exhibit its usual increase in size, may not store its full food reserve for the first half of the year to follow and so not only grow poorly in general, but also not be able to exhibit the usual relative growth of the central nervous system during the season which follows.

It is hardly necessary to elaborate these relations, enough having been said to indicate why frogs taken at the same date and in the same locality, may exhibit wide differences in the relative weight of the central nervous system. What we find in the case of any frog probably depends in large measure on the external conditions to which that individual has been subjected, not only during the season in which it was caught, but also during the season which preceded. If one turn back therefore to table 1, it appears that in the Chicago series the minimal values of  $C$  vary irregularly from month to month; suggesting that some of the individuals grew very little—as this would be the readiest explanation of the absence of systematic changes—while the maximal values show more consistent changes, tending to follow the mean.

On the other hand, in the case of both the Minnesota and Brandywine frogs, both the minimal and maximal values tend to

follow the mean values more regularly; Minnesota, table 5, Brandywine, table 7.

#### SUMMARY

From the foregoing discussion, the following conclusions are drawn:

1. The relative weight of the central nervous system of the frog, *Rana pipiens*, changes during the active season, and such a change is probably characteristic for other species of frogs with like habits.

2. The relative weight of the central nervous system is low at the time of emergence, high in the midsummer (July) and low again at the time of hibernation. During hibernation it remains nearly constant. In the formula used to express the weight of the central nervous system, the absolute value of  $C$  is characteristic for the station from which the frogs come.

3. The range from minimum to maximum in the value of  $C$  is about 13 per cent, rising 7 per cent from the end of March to the end of April, 4 per cent more from the end of April to the end of May, and 2 per cent more from the end of May to the first of July, remaining stationary in July and then in reverse order falling month by month at a similar rate to the end of October.

4. This variation in the relative weight according to season is due to lack of coincidence between the growth of the central nervous system and the growth of the entire body.

5. In frogs from one to four years old, the body weight more than doubles during each active season. The precise form of the curve representing this body growth is not known.

6. The growth of the central nervous system is precocious in relation to that of the body, but in the absence of direct observations on the growth of the body, the form of the curve can only be indirectly determined as shown in chart 5.

7. During the active season, the percentage of water in the entire frog falls slightly from spring to summer and rises again from summer to autumn. These changes seem to be due to the combined effects of advancing age and varying food supply.

The conclusions just given apply primarily to the interpretation of the preceding observations, but secondarily they also

bear on the phenomena of growth as shown by vertebrates in general. The curve for the growth of the central nervous system of the frog as given in chart 5 has the general character of the corresponding curve for a mammal; but as is evident, this curve in the case of the frog must repeat itself from year to year, so that if we should plot the entire curve for the span of life, rather than for a single season, it would be represented by a sinuous ascending line in which the sinuosities would probably diminish towards the upper end.

If we turn now to general body growth, which is closely correlated with that of the central nervous system, it appears that the poikilothermous vertebrates as a group must show a seasonal variation in growth in all latitudes where there is any marked seasonal change, and that the phenomena of hibernation, with the concomitant effects with which we have to deal, represent merely a special case of this seasonal variation.

If now we pass up the vertebrate scale we find in the temperate zones both hibernating mammals, as well as those in which the seasons seems to produce marked nutritional modifications, and finally we have Malling-Hansen's observations ('86) on Danish children from 9 to 15 years of age which show that the growth in stature is mainly in the third of the year between the middle of April and the middle of August, while the third comprised between the middle of August and the middle of December is the one in which they gain nine-elevenths of their annual increase in body weight. This leaves the remaining third from mid-December to mid-April, *i.e.*, late winter and early spring, as the one in which very little growth of any sort occurs. This seems to link the rhythmic growth in the frog with that in man. To be sure there are at present but very few data available, but such as we have suggest that within the annual cycle we should expect even in the higher vertebrates a distinct rhythm corresponding to the responses of the poikilothermous vertebrates, and still exhibited even by the group in which the regulation of temperature has been more or less completely attained.

Just one point more. The rate of growth in the frog, more than doubling its body weight for three successive years (as far as we have observations) shows that the rate in the frog does not

fall off with anything like the rapidity that it does in man and some other mammals. This difference suggests a number of questions to answer which it will be necessary to take up the study of growth in hibernating mammals.

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## THE PHYSIOLOGY OF CELL-DIVISION

### IV. THE ACTION OF SALT SOLUTIONS FOLLOWED BY HYPERTONIC SEA-WATER ON UNFERTILIZED SEA-URCHIN EGGS AND THE RÔLE OF MEMBRANES IN MITOSIS

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THREE FIGURES

#### INTRODUCTION

During the summer of 1909 at Woods Hole I found that membrane-formation and cleavage, leading in a small proportion of cases to the production of blastulae, could be induced in unfertilized eggs of *Asterias* and *Arbacia* by temporary exposure to isotonic solutions of various neutral salts.<sup>1</sup> Salts of sodium and potassium were chiefly employed, including chloride, bromide, nitrate, iodide and sulphocyanate; last summer chlorate was also used. In the case of *Asterias* all of these were found to induce membrane-formation and cleavage in a large proportion of eggs. With *Arbacia*, however, only iodide and sulphocyanate showed a corresponding degree of effectiveness; nitrate had well-marked though less decided action, chlorate produced little and bromide still less effect, while chloride was almost entirely inactive; sodium acetate was found to act like chloride. The order of relative effectiveness of the salts, ranged according to the anions, is as follows:  $\text{COO CH}_3$  and  $\text{Cl} < \text{Br} < \text{ClO}_3 < \text{NO}_3 < \text{I}$  and  $\text{CNS}$ . This order is also that of relative activity in freeing pigment from the eggs; a perceptible loss of pigment occurs within two or three minutes in iodide and sulphocyanate solutions and more slowly in solutions of the other salts. The exit of pigment

<sup>1</sup> R. S. Lillie: American Journal of Physiology, 1910, vol. 26, p. 106.

is to be regarded as a consequence of increased surface permeability;<sup>2</sup> hence the inference naturally follows that the critical change in the egg, to which membrane-formation and the initiation of cleavage are due, is a well-marked and rapid increase in the permeability of the plasma-membrane.

The differences between the above salts are to be regarded as dependent on differences in the physico-chemical characteristics of their anions; the most effective salts, iodide and sulphocyanate, are those whose anions have greatest action in altering the state of aggregation of proteins and other negative colloids (including lipoids) in the direction of increased dispersion. The order of physiological activity thus corresponds to the order of relative action on colloids. The effect is therefore to be attributed to the action of the salt on the colloids of the plasma-membrane, whose permeability is thus altered at a rate and to a degree determined by the physico-chemical activity of the anion.

A similar agreement between the order of relative physiological activity of neutral salts of alkali metals and the order of relative action on colloids has been observed in a variety of cases. From his experiments on the influence of isotonic solutions of these salts on the demarcation current of muscle Höber concludes that

<sup>2</sup> It appears out of the question to assume, as apparently some have done, that *none* of the pigment is free to diffuse within the cell, that it is all combined or adsorbed in solid granules or chromatophores. Granting that this is the case for part of the pigment, it is always necessary to assume the existence of an equilibrium between the adsorbed pigment and that in true solution and capable of diffusion. The homogeneous distribution of the pigment in *Arbacia* eggs in the space between the nuclear and plasma membranes is practically a proof of its free diffusibility; another and more cogent proof, is that it is liberated from the egg by *any* substance, irrespective of its chemical nature, that alters the surface protoplasm. That traces of saponin, mercury or silver salts, acid, alkali, as well as the above neutral salts, should all have the same effect, proves that a specifically chemical alteration is not the ground of the diffusion of the pigment to the exterior. The exit of pigment, besides being the most convenient index of a decided increase of permeability, has the advantage over indexes based on the use of foreign substances, that a substance normally present in the cells is concerned. There is always the doubt, in estimating the permeability by the addition to the medium of a foreign substance like alkali, that the latter produces abnormal alterations in permeability and thus gains entrance. Final conclusions should be based on the use of several methods serving for mutual control. The possibility of selective permeability must also be taken into account.

the ions act primarily on the plasma-membrane, altering the permeability of the latter, with corresponding changes in the electrical and other physiological properties of the tissue.<sup>3</sup> The influence of salts in initiating cell-division is, on the present theory, an instance of an essentially similar kind.

Since in *Arbacia* eggs only the most active salts, iodide and sulphocyanate, and to a less degree nitrate, have decided action in inducing cleavage, while all increase the surface-permeability, though at different rates, it may be inferred that the *rate* of permeability-increase ( $\frac{dp}{dt}$ ) must exceed a certain critical minimum in order that cleavage may be started; hence the more slowly acting salts—chloride, bromide, and acetate—are practically ineffective with these eggs. In *Asterias* eggs, on the other hand, the permeability of the plasma-membrane is more readily increased, and all of the above salts<sup>4</sup> produce membranes and start cleavage. Different eggs thus vary in their response to a given method of treatment according to the specific peculiarities of their plasma-membranes. In *Asterias* the plasma-membranes are evidently more susceptible to changes of permeability than in *Arbacia*. The former eggs are less stable, less uniform in behavior, and less tenacious of life; and these peculiarities are undoubtedly related to the greater ease with which they may be induced by artificial means to cleave and develop. It is self-evident that plasma-membranes which are highly impermeable and whose permeability is increased with difficulty must isolate the protoplasmic complex more completely from its surroundings than membranes of a more permeable and less stable kind, and that a more radical change in external conditions will be required to influence the metabolic or other processes in eggs thus enclosed. It is, I believe, for this reason that *Arbacia* eggs are relatively resistant and unresponsive as compared with *Asterias* eggs. Analogous considerations apply in other cases; hence the prob-

<sup>3</sup> Cf. Höber: *Physikalische Chemie der Zelle und der Gewebe*. Engelmann, Leipzig, 1906, pp. 217 seq., 370 seq. Further references in this book.

<sup>4</sup> Possibly excepting acetate whose action on starfish eggs I have not yet tried. I am confident, however, from the effects produced by chloride, that acetate will be found to have well-marked action on *Asterias* eggs.

lem of inducing parthenogenesis in unusually resistant eggs resolves itself into a choice of means by which the requisite rapid and reversible increase of permeability may be effected. I have considered the conditions in *Asterias* in more detail elsewhere.<sup>5</sup> In the present paper the description will be confined to experiments with *Arbacia* eggs.

The following experiments were suggested by the foregoing theoretical considerations. If the essential action of the above salts, acting as parthenogenetic agents, is to cause a rapid initial increase of permeability, membrane-formation and the initiation of cleavage should be prevented by the addition of other salts that oppose the permeability-increasing action. In studying the toxic and antitoxic action of various salts on the pigmented larvæ of *Arenicola* I had observed that the effect produced by an antitoxic salt like calcium chloride in diminishing the toxicity of pure solutions of sodium salts was always associated with a prevention of the marked and rapid increase of permeability,—indicated by loss of pigment—which the pure solution typically produces.<sup>6</sup> Now it seems clear that any marked and unchecked increase of permeability, sufficient to destroy the normal osmotic equilibrium and allow abnormal diffusion of substances into and out of cells, must derange and eventually destroy the chemical organization of the latter. It is, I believe, essentially for this reason that pure solutions of sodium salts are highly toxic; but if to such solutions small quantities of calcium or other favorable salts are added, the permeability remains normal or undergoes only gradual alteration so that the cell is enabled to retain its normal properties for greatly prolonged periods. The antitoxic salt thus acts by preventing or checking increase in permeability. I have already mentioned that pure isotonic solutions of neutral sodium or potassium salts acting on unfertilized *Arbacia* eggs produce a similar increase in permeability with loss of pigment, which is most rapid in iodide and sulphocyanate solutions; this effect, together with the associated toxic action, is greatly checked by adding a little

<sup>5</sup> American Journal of Physiology, 1911, vol. 27, p. 289.

<sup>6</sup> American Journal of Physiology, 1909, vol. 24, pp. 23–25. Other observations not yet published confirm these results.

calcium to the solution. It is therefore to be expected that the initiation of cleavage by such solutions, if dependent on rapid increase of permeability, will also be prevented by the addition of calcium. This has been found to be the case.

#### EXPERIMENTAL

In the following experiments unfertilized eggs of *Arbacia* were exposed for five or ten minutes to isotonic solutions of neutral salts of sodium and potassium. The effects produced by pure solution and by solutions containing calcium chloride were studied and compared. The salts used were chlorate, nitrate, iodide, and sulphocyanate.<sup>7</sup> The effect of after-treatment with hypertonic sea-water and cyanide-containing sea-water was also studied.

The procedure was the same as in my former experiments. In each series eggs were exposed for five and ten minutes to (a) the pure solution of the salt and (b) to the same solution *plus* a definite quantity of  $\frac{M}{2}$   $\text{CaCl}_2$ : usually 12.5, 15, or 25 cc.  $\frac{M}{2}$   $\text{CaCl}_2$  was added to 250 cc. of the pure salt solution. The concentration of the latter was 0.55 M.

The essential results of these experiments may be described in a few words. Eggs exposed for five or ten minutes to pure isotonic sodium or potassium iodide or sulphocyanate solutions and then returned to sea-water undergo the series of changes described in my former paper. A large proportion, in favorable experiments practically all, form fertilization membranes, usually thin and close to the egg-surface; two or three hours later the eggs are found for the most part to be more or less irregular or amœboid in form and in some cases subdivided into several cells. These cleavages are almost always irregular and much slower than nor-

<sup>7</sup> Chloride and bromide were omitted as practically inactive. The salts, it will be noted, are all salts of strong monobasic acids; the solutions were thus neutral in reaction in all cases. Since traces of alkali may produce membranes it is important to make sure of this. Commercial sulphocyanate preparations are often distinctly alkaline; those used in the present experiments were neutral to phenolphthalein. The OH-ion concentration was thus below  $10^{-8}$  N—less than that of the Woods Hole sea-water. The salts used were Kahlbaum's and Baker's analysed.

mal. Usually a small proportion of such eggs develop to the blastula stage; such blastulae are, as a rule, feeble and more or less abnormal; the proportion so developing is variable and always small—a fraction of one per cent. The four salts NaI, NaCNS, KI, KCNS all produce essentially the same result; NaCNS however has proved somewhat less effective as well as less toxic than the others. Nitrate produces membranes and change of form in a smaller though still considerable proportion of eggs, varying from ten to fifty per cent. Chlorate is less effective than nitrate and shows little action, though decidedly more than bromide. The differences between sodium and potassium salts have proved indecisive.

Even in the most favorable experiments the great majority of eggs treated in the above manner merely undergo some change of form or show a few irregular cleavages and then die and disintegrate. Next day they are typically found dead, coagulated, and decolorized, with a few exceptions. Brief exposure to isotonic iodide and sulphocyanate solutions has in fact an effect upon the eggs essentially similar to that produced by weak solutions of fatty acids or other membrane-forming substances. It is significant that after twenty-four hours the eggs in these cultures are typically free from pigment, indicating that the permeability has remained permanently greater than normal. The toxic effect of the treatment is, I believe, due essentially to the persistence of this condition of abnormally increased permeability. Cytolysis hence follows in course of time.

Invariably the eggs treated with the pure and those treated similarly with the calcium-containing solutions present a striking contrast. The above effects are in the great majority of eggs entirely prevented by the presence of calcium chloride in the proportions named below. The eggs remain round and unaltered—with a few exceptions, especially in sulphocyanate solutions—showing no sign of membrane-formation or change of form, and next day almost all remain living and on fertilization with spermatozoa largely develop into normal larvæ. It should be added that the action of sulphocyanate is less completely counteracted by calcium than that of nitrate and iodide, though with all salts the above contrast is strongly marked.

That the calcium prevents the rapid increase of permeability caused by the pure solution may readily be proved. I have already described experiments of this kind.<sup>8</sup> Eggs placed in pure sodium iodide or sulphocyanate solutions lose pigment rapidly, in nitrate solution more slowly—within several minutes,—and still more slowly in solutions of the other sodium salts. Calcium-containing solutions of the same salts, in which the same quantities of eggs have been placed, remain uncolored for hours, or in the case of the more weakly active salts for a day or more. In the pure solutions eggs are rapidly killed,<sup>9</sup> i.e., lose the power of developing on fertilization; in calcium-containing solutions, on the other hand, they preserve their normal properties for greatly prolonged periods, varying in duration with the salts, according to the specific toxicity of the latter. The rapid increase in permeability is thus prevented at the same time as the toxic action is diminished; hence the preservation of the normal properties of the protoplasm, i.e., the antitoxic action exercised by calcium, appears to be dependent on the maintenance of a normal or approximately normal state of surface-permeability. The cleavage-initiating action, with which is also associated a rapid increase of permeability, is similarly prevented by the calcium. The inference that the increase of permeability is the essential or critical change in normal or parthenogenetic fertilization thus seems highly probable.

Several years ago Loeb discovered that while only a few eggs at best developed to a larval stage after simple membrane-formation by fatty acid or otherwise—the great majority dying early—the proportion undergoing favorable development was greatly increased by subsequent treatment with hypertonic or cyanide-

<sup>8</sup> American Journal of Physiology, 1911, vol. 27, p. 289.

<sup>9</sup> Different eggs vary in their resistance to the destructive action of pure isotonic solutions of neutral alkali metal salts. Unfertilized *Arbacia* eggs may survive several hours immersion in isotonic NaCl solution; *Asterias* eggs are killed comparatively rapidly, while *Strongylocentrotus* eggs may remain living in this solution for 48 hours (cf. J. Loeb: *Biochemische Zeitschrift*, 1906, vol. 2, p. 84). The relative ease with which parthogenetic development may be induced corresponds to this difference. *Strongylocentrotus* eggs are the least responsive (Loeb: *Chemische Entwicklungsregung*, p. 67), *Asterias* the most.

containing sea-water.<sup>10</sup> This remarkable result, which he ascribed to a modification of oxidation-processes in the egg, is seen also in eggs treated with isotonic iodide or sulphocyanate solutions, and, to a less extent, with nitrate or chlorate. The physiological basis of this profound change in the developmental capability of eggs with artificially formed membranes is still largely obscure. That oxidations play a part seems clear from Loeb's admirable experiments; I am inclined, however, to attribute also a fundamental significance to alterations of surface permeability. If the above view as to the nature of the primary change in fertilization be well founded, it is clear that the increased permeability resulting from the action of the membrane-forming agency can be only temporary if favorable development is to follow, since a prolonged loss of semi-permeability must lead to the cytolytic dissolution of the egg-protoplasm. In fact the dead eggs, after the above salt-treatment, are always found laked or depigmented on the next day, while the living eggs retain the pigment. The initial increase of permeability must thus under physiological conditions be temporary and not too lasting; hence the inference seems unavoidable that one essential effect of the after-treatment with hypertonic sea-water is to restore the normal permeability. This hypothesis is an almost necessary corollary of the foregoing considerations. The cytolytic action following simple membrane-formation and due apparently to the persistence of an abnormally increased permeability is in fact prevented by subsequent treatment with hypertonic sea-water or cyanide.<sup>11</sup> A demonstrable consequence of the after-treatment is thus to restore a normal condition of permeability, and that this is the essential action seems highly probable. I have discussed this possibility in another paper to which I refer those who may be interested in this question.<sup>12</sup>

The following experiments will illustrate the foregoing general description. Unfertilized eggs of *Arbacia* were exposed for periods of five and ten minutes to (1) the pure salt solution and (2) the same solution *plus* a small proportion of  $\frac{M}{2}$   $\text{CaCl}_2$ . Part of the

<sup>10</sup> Cf. *Chemische Entwicklungserregung*, chapter 8, p. 60.

<sup>11</sup> Cf. Loeb: *loc. cit.*, chapter 10, p. 77.

<sup>12</sup> *American Journal of Physiology*, 1911, vol. 27, pp. 295, 304.

eggs thus treated were left in sea-water without further treatment; a second part after a brief interval were transferred to hypertonic sea-water for thirty minutes, and then returned to sea-water; a third part were left for varying periods in sea-water containing  $\frac{M}{2000}$  KCN.

TABLE 1

*July 6, 1910. Unfertilized eggs of Arbacia punctulata were divided into four equal lots in finger bowls, the sea-water was removed as far as possible, and solutions A, A(+Ca), B, B(+Ca) added respectively to each lot. After five minutes the eggs were returned to sea-water. Part 1 remained in sea-water; Part 2 were transferred after several minutes to hypertonic sea-water (250 cc. sea-water plus 25 cc. 2.5 M NaCl); and Part 3 were transferred after about ten minutes into sea-water containing KCN to  $\frac{M}{2000}$  concentration, from which they were returned to sea-water after varying intervals. The results were as follows:*

A. Eggs in 250 cc. 0.55 M NaI for 5 minutes; thence transferred to sea-water. Further treatment as follows:

1. *Eggs left in sea-water.* Almost all form membranes and undergo form-change or irregular cleavage. Next day the great majority are dead, coagulated, and depigmented. A few blastulae—two or three—found in several hundred eggs.

2. *Eggs left in sea-water 5 minutes; thence transferred to hypertonic sea-water for 30 minutes; returned to sea-water.* A large proportion of eggs form active blastulae (ca. 50 per cent).

3. *Eggs left in sea-water 11 minutes; thence to  $\frac{M}{2000}$  KCN in sea-water; portions returned to sea-water after following intervals.*

a. *2 hours.* A small proportion form blastulae. Decided increase over 1.

b. *3 hours.* A fair proportion of blastulae, more than in 3a though few compared with 2.

c. *4½ hours.* Like 3b: a fair proportion of larvae.

A(+Ca.) Eggs left for 5 minutes in 250 cc. 0.55 M NaI plus 15 cc.  $\frac{M}{2}$  Ca Cl<sub>2</sub>; thence to sea-water. Part left in sea-water, part treated with hypertonic sea-water.

1. *Eggs left in sea-water.* Marked contrast to A1. Almost all eggs remain round, uncleaved, and unaltered. A small proportion undergo irregular change of form and break down. No larvæ.

Next day, after 22 hours, the eggs were fertilized with spermatozoa; practically all developed into swimming larvae.

2. *Eggs left in sea-water 7 minutes; thence to hypertonic sea-water for 30 minutes; returned to sea-water.* The great majority of eggs remain unaltered. A small proportion are broken down next day and a few feeble blastulae are present.

On fertilization next day (after 22 hours) most eggs form larvae, but a smaller proportion than in Lot 1 swim at the surface of the water.

B. Eggs in 0.55 M KCNS for 5 minutes; thence to sea-water. Three lots treated as follows:

1. *Egg left in sea-water.* Almost all form membranes and undergo change of form or irregular cleavage. Next day almost all are dead, coagulated, and depigmented. A few blastulae; two or three found in several hundred eggs.

2. Eggs left in sea-water 5 minutes; thence to hypertonic sea-water for 30 minutes; returned to sea-water. A large proportion (ca. 50 per cent) form active blastulae.

3. Eggs left in sea-water 11 minutes; thence to  $\frac{M}{2000}$  KCN; thence to sea-water after the following intervals.

a. 2 hours. Blastulae few, but more than in B1.

b. 3 hours. Blastulae more numerous than in 3a, but few as compared with B2.

c.  $4\frac{1}{2}$  hours. A small proportion of blastulae.

B (+Ca). Eggs left for 5 minutes in 250 cc. 0.55 M KCNS + 15 cc.  $\frac{M}{2}$  CaCl<sub>2</sub>. Thence returned to sea-water. Part left in sea-water, part treated with hypertonic sea-water.

1. Eggs left in sea-water. Marked contrast to B1. Great majority remain unchanged. A small proportion (more than in A (+Ca) 1) show membranes and irregular change of form; no larvae.

After 22 hours eggs are fertilized: almost all develop normally; after 2 days the dish is full of vigorous plutei.

2. Eggs left in sea-water 7 minutes; thence to hypertonic sea-water 30 minutes; returned to sea-water. Eggs are mostly unaltered next day, but the proportion broken down is higher than in the similar experiment with NaI. A considerable number of blastulae are formed.

On fertilization after 22 hours most eggs form larvae, but these are largely abnormal; few surface swimmers.

Control experiments. Unfertilized untreated eggs remain unchanged. Fertilized eggs all develop normally.

The above experiments show that brief exposure to pure isotonic sodium iodide or potassium sulphocyanate solutions has the effect of producing fertilization-membranes and initiating change of form or cleavage in the majority of eggs; but that if left in sea-water after such treatment nearly all die in an early stage of development; after eighteen hours such eggs appear broken down, coagulated and depigmented, proving the persistence of a condition of increased permeability. Such persistence is in itself sufficient to account for the destructive effect of the treatment. On the other hand, if the eggs are treated, soon after return from the salt-solution to sea-water, with hypertonic sea-water for thirty minutes, a large proportion—in favorable experiments the great majority—form larvae, many of which swim actively at the surface of the water and develop into normal plutei. The rate of development of such larvae is fully equal to the normal. The early stages, however, always show certain characteristic abnormalities; the fertilization membranes are thinner than normal, the cleavage is usually irregular, and the blastomeres tend to have a more rounded form and to cohere less closely than in sperm-

fertilized eggs. These abnormalities can in all likelihood be largely removed by further experimentation. It is however scarcely to be expected that any artificial treatment will produce such uniform results as natural fertilization, since the uncontrolled variables are necessarily more numerous in the former case. The essential facts are that the after-treatment with hypertonic sea-water prevents the otherwise resulting cytolysis—an effect due probably to a restoration of the normal conditions of permeability—and enables the eggs to continue their development to an advanced larval stage. Cyanide has a similar, though in my experience less favorable, action.

Similar exposure to the calcium-containing solutions leaves the great majority of eggs essentially unaltered; after remaining twenty-four hours in sea-water they appear round and unaltered like normal untreated eggs, and on fertilization with spermatozoa develop normally. A certain small proportion, however, may form membranes under the influence of such solutions; this proportion is higher with sulphocyanate than with iodide. Thus the calcium does not completely suppress though it greatly checks the action of the iodide or sulphocyanate. Experiments on the permeability-increasing action of these salt solutions confirm this conclusion; pigment is slowly liberated in calcium-containing iodide and sulphocyanate solutions (more slowly in the former than the latter), though the contrast to the rapid action of the pure solution is a striking one. Apparently in a small proportion of eggs the calcium-containing solutions may effect an increase of permeability rapid enough to start development.

Hence it is usually found that after-treatment with hypertonic sea-water produces a small proportion of larvae from such eggs. This proportion is of course much smaller than in eggs treated previously with the pure solution, but in the case of KCNS may be considerable. The majority of eggs, as already stated, remain unchanged, and on subsequent sperm-fertilization develop into larvæ. It should be noted that the above hypertonic sea-water acting on normal unfertilized *Arbacia* eggs for thirty minutes has little or no action; a few eggs form membranes and a few undergo breakdown and an occasional larva may be formed, but the

vast majority undergo no apparent change and when fertilized next day develop into normal larvae.

The calcium-containing solutions are thus not quite indifferent in their action. It is probably not possible to produce a completely indifferent or 'physiologically balanced' salt-solution containing a high proportion of either of the above salts, though it is easier to obtain well marked antitoxic action with iodide than with sulphocyanate.<sup>13</sup> It is well known that addition of appropriate quantities of calcium, magnesium, and potassium salts to pure solutions of sodium chloride or bromide produces a practically indifferent medium; but the specific toxicity of many salts, including the above, is too great to be thus fully counteracted. The case of *Asterias* eggs, in which brief exposure to pure isotonic sodium chloride solutions produces membranes and initiates development, illustrates the essential nature of the conditions perhaps more clearly than the above; addition of calcium chloride greatly checks the membrane-producing and permeability-increasing action of the pure solution, and with it the cleavage-initiating action, but further addition of magnesium and potassium salts, as in van't Hoff's solution, is required to produce a medium which is completely indifferent.

Treatment with potassium iodide or sodium sulphocyanate, followed by hypertonic sea-water as above, gives similar results. The following experiments will illustrate. Eggs were placed for five minutes in each of the following solutions: (A) 0.55 M NaCNS, (B) a mixture of 250 cc. 0.55 M NaCNS + 25 cc.  $\frac{M}{2}$   $\text{CaCl}_2$ , (C) 0.55 M KI, (D) 250 cc. 0.55 M KI + 25 cc.  $\frac{M}{2}$   $\text{CaCl}_2$ ; they were then returned to sea-water. In each case part of the eggs were allowed to remain in sea-water; the rest were exposed to hypertonic sea-water for 30 minutes and then replaced in sea-water. Treatment with the pure solution alone (A and C) produced membranes in a majority of eggs, of which a small proportion (one in several hundred) formed blastulae; while of the eggs treated also with hypertonic sea-water the majority formed larvae, many of which swam vigorously at the surface and were apparently quite normal. Exposure to the calcium-containing solutions (B

<sup>13</sup> Cf. my results with ciliated cells: American Journal of Physiology, 1906, vol. 17, pp. 104, et seq.

and *D*) left the great majority of the eggs practically unaltered, as shown by absence of change (no larvae) and by normal development on fertilization with sperm next day; eggs after-treated as above with hypertonic sea-water also remained unchanged for the most part, but a small proportion yielded larvae—smaller than in the experiments of table 1, a difference due probably to the higher calcium-content of the solutions. In another series of similar experiments, in which potassium iodide and potassium sulphocyanate were compared, the same result was obtained. In this series also the action of the sulphocyanate was less completely inhibited than that of the iodide by the presence of calcium. Treatment with hypertonic sea-water after the calcium-containing sulphocyanate solution gave a considerable number of larvae, though few compared with those obtained with the pure solution followed by hypertonic sea-water.

Results similar to the above were obtained in ten other series of experiments with sulphocyanate and iodide solutions. With sodium and potassium nitrate and sodium chlorate a similar increase in the proportion of developing eggs followed after-treatment with hypertonic sea-water; but the effect was less pronounced, and the proportion of larvae was never high. Nitrate was more favorable than chlorate. Hypertonic sea-water thus has well-marked action only when the initial treatment with the isotonic salt solution has produced a decided change in the eggs.

*Time-relations of the above treatment.* It was found that the after-treatment with hypertonic sea-water produced the most favorable results if the eggs were brought into this medium within a fairly definite interval—from ten to fifteen minutes—after the treatment with isotonic salt-solution. This is illustrated by table 2.

These experiments indicate that the most favorable interval between the return from the salt solution to sea-water and the exposure to the hypertonic sea-water is from ten to fifteen minutes; an interval of twenty minutes seems already above the optimum; other experiments in which the interval was shorter—five to seven minutes—gave somewhat fewer larvae than in Experiments 1 and

TABLE 2

*August 22, 1910. Eggs were exposed to 0.55 M KCNS for 5 minutes, then returned to sea-water, and after the following intervals transferred to hypertonic sea-water in which they remained 30 minutes. The results were as follows:*

NO.	INTERVAL BEFORE TRANSFER TO HYPER- TONIC SEA-WATER	RESULT
	<i>minutes</i>	
1.....	10	From one-half to two-thirds of the eggs form larvae; many vigorous surface-swimmers
2.....	20	Also shows a large proportion of active larvae though apparently fewer than in Experiment 1
3.....	30	Decidedly fewer larvae than in experiments 1 and 2; about 5 per cent of the eggs form larvae; a good many surface-swimmers
4.....	40	Still fewer eggs form larvae— <i>ca.</i> 1 per cent; a few surface-swimmers

2 of table 2, though the proportion was still high.<sup>14</sup> It is clear that during the period succeeding the return from the salt-solution to sea-water the egg undergoes a progressive series of changes of such a kind that the favorability of the response to the hypertonic sea-water undergoes decided decrease after the lapse of a certain interval, which, in these eggs, appears to be about fifteen or twenty minutes at normal summer temperature (20° to 22°). The nature of these changes can at present only be inferred; but if we accept the view proposed above that the effect of the hypertonic sea-water is to bring the permeability—which has been increased by the salt-solution—again toward normal, the conditions become partly intelligible on the assumption that the initial state of increased permeability cannot be prolonged beyond a certain period without

<sup>14</sup> Loeb's experiments (with Moore: *loc cit.*, pp. 78, 79) also show that too long an interval must not be allowed to elapse between membrane-formation and transfer to hypertonic sea-water. The time-relations in these experiments were, however, decidedly different from the above. Eggs brought into hypertonic sea-water two hours after membrane-formation showed a high proportion of favorable development. The difference, I believe, is to be explained as due partly to the more resistant character of *Strongylocentrotus* eggs, and partly to the fact that the temperature of the sea-water in Loeb's experiments was low (12°). The favorable interval between membrane-formation and exposure to hypertonic sea-water would undoubtedly be several times shorter at a temperature 10° higher, as in my experiments described above.

injury to the egg. In normal fertilization the evidence indicates that the condition of increased permeability lasts for a certain time—roughly fifteen minutes—after the contact of the spermatozoon,<sup>15</sup> and is then succeeded by a period of normal permeability (like that of the unfertilized egg) which lasts until the appearance of the cleavage furrow about half an hour later. If the variations in the state of permeability could be graphically represented by a curve (with degree of permeability as ordinates and time as abscissae) the latter would probably rise rapidly immediately after the contact of the spermatozoon and fall again toward normal soon after the spermatozoon had completed its entrance; it would then rise again temporarily at the time of the first cleavage, and similarly with each succeeding cleavage. Presumably any parthenogenetic treatment—if the best results are to be attained—should approach the normal in the time-relations of the permeability-changes which it produces.

The optimum duration of the exposure to hypertonic sea-water after membrane-formation by salt solutions is about twenty-five or thirty minutes at normal summer temperature (20° to 23°); twenty minutes is too brief and thirty-five minutes too long. If we assume that the next sudden change in external conditions, the transfer from hypertonic to normal sea-water, momentarily increases permeability, we should expect that the closest approach to the normal conditions would be obtained if the time of this transfer were to coincide with the time when the permeability *normally* undergoes a second increase, i.e., the time of appearance of the first cleavage-furrow. The above time-relations indicate that this is the case, and the agreement may be more than a mere coincidence. These considerations suggest that the processes occurring in the egg during the stay in hypertonic sea-water are essentially similar, both in rate and character, to those taking place in normally fertilized eggs during the half-hour preceding the appearance of the cleavage-furrow. That they are largely oxidative in their nature is indicated by Loeb's experiments, which show that the hypertonic sea-water must contain oxygen if it is to produce its characteristic effect.

<sup>15</sup> Cf. my recent paper on the present subject, *American Journal of Physiology*, 1911, vol. 27, p. 295.

The fact that the optimum duration of exposure to cyanide-containing sea-water is much longer than thirty minutes—in fact, several hours—may seem inconsistent with this interpretation. Cyanide, however, produces its effect by checking oxidations and so slowing the entire metabolism of the egg. Hence it is not surprising that the chemical processes within the egg proceed too slowly in cyanide-containing sea-water to impair the developmental power, even after a stay of some hours. In hypertonic sea-water, on the contrary, oxidations continue unchecked, as Loeb as shown; and the condition of the egg undergoes progressive change of such a kind that return to sea-water within a comparatively brief interval is necessary if normal development is to continue. If the oxidations are suppressed during the stay in hypertonic sea-water the latter has little or no action; the injury normally resulting from a too prolonged exposure to this medium is also prevented.<sup>16</sup>

It should not be overlooked, in considering the mode of action of hypertonic solutions, that they appear to increase the rate of oxidations in the egg;<sup>17</sup> and although they may do this indirectly by altering permeability, there is no direct evidence as yet that this is the case. Loeb has found hypertonic sea-water ineffective in the absence of oxygen or in the presence of cyanide,<sup>18</sup> and concludes that the alteration in rate and character of intracellular oxidations is the essential change involved. But just how hypertonic sea-water can modify oxidations remains obscure. The fact that it checks or prevents the cytolysis which otherwise succeeds the temporary treatment with pure salt-solution has been interpreted above as indicating an action on permeability essentially similar to that exerted by calcium in antitoxic action; but I have been unable as yet to produce the effects of hypertonic sea-water by the use of sea-water containing an increased proportion of calcium or magnesium. I have elsewhere adduced evidence indicating that anaesthetic or narcotic action is essentially a consequence of a *decrease* in the normal permeability.<sup>19</sup> If this

<sup>16</sup> Loeb: *Chemische Entwicklungserregung*, pp. 51 seq.

<sup>17</sup> Warburg: *Zeitschrift für physiologische Chemie*, 1908, vol. 57, p. 1.

<sup>18</sup> J. Loeb: *Chem. Entw.*, also *Biochemische Zeitschrift*, 1906, vol. 1, p. 183.

<sup>19</sup> Cf. *American Journal of Physiology*: 1909, vol. 24, pp. 14, 459; *ibid.*, 1910, vol. 26, p. 114. At least a decreased susceptibility to increase of permeability must

be true it might be expected that weak solutions of anaesthetics could be substituted for hypertonic sea-water. Experiments now being conducted (June 1911) indicate that this is in fact the case with starfish eggs. Loeb has found chloral hydrate—a typical anaesthetic—to have an action on the eggs of *Strongylocentrotus*, after artificial membrane-formation, similar to that of potassium cyanide.<sup>20</sup> This fact accords with the foregoing interpretation. My experiments of this summer (1911) are at present too incomplete for further description.

#### THEORETICAL

According to the view put forward in the present and preceding papers alterations in the permeability of the limiting membranes of the cell are events of critical importance in the process of cell-division. An important controlling or directive rôle is thus ascribed to the membranes; it remains to consider more fully the manner in which changes in the permeability of these structures can be the condition of changes so extensive as those concerned in cell-division and development.

Development in all cases involves the transformation of a quantity of inert or non-living material into the characteristically organized and active adult animal or plant. It is thus largely a matter of definitely directed constructive metabolism. Since mitotic cell-divisions are intimately associated with the developmental process in all metazoa, one of the first steps in the investigation of the physiology of development must be to determine the nature of the metabolic processes in mitosis and of the conditions controlling them.

be assumed. Thus *Arenicola* larvae brought from sea-water into pure isotonic NaCl solutions contract strongly, while at the same time the cells rapidly lose pigment. If, on the other hand, the organisms are brought from sea-water into isotonic magnesium chloride or sulphate solution, all contraction ceases and the animals remain rigid and extended, with no sign of muscular movement or loss of pigment; i.e., a typical anaesthetic action is seen. If the larvae, after an interval of some minutes, are then transferred to pure NaCl solution, no contractions, or at most very slight ones, result and there is no loss of pigment. In other words, the susceptibility to stimulation and increase of permeability is greatly decreased by the magnesium salt. The same is true of chloroform and ether in appropriate concentrations.

<sup>20</sup> J. Loeb: *Chemische Entwicklungserregung*, p. 70.

The question which I propose briefly to discuss in this section of the present paper is as follows: What relations can alterations in the permeability of the cell-membranes have to the mitotic process? Perhaps the most evident answer to this question, and one probably in large part correct, is the following: since (1) cell-division typically involves growth, which implies the incorporation of surrounding food materials, and since (2) the plasma-membrane during the resting condition of the cell appears virtually impermeable to many diffusible substances essential to the cell, such as sugars, amino-acids, and neutral salts, periods of increased permeability are necessary in order to provide for the ready entrance of outside materials into the cell. The increase in the permeability of the nuclear membrane, leading typically to complete dissolution of this structure, is necessary to permit of free interchange between nuclear and cytoplasmic regions.<sup>21</sup> In general, increased permeability of the membranes facilitates or perhaps for the first time renders possible transfer of certain materials, particularly those normally kept apart by the membranes, between the regions thus separated.

The import of dissolved food-materials, however, like the separation of dissolved substances in secretion (*e.g.*, urea by the kidney), is not a mere matter of passive diffusion, but requires the performance of work; so that the simple assumption of an increase in permeability is insufficient entirely to account for the conditions. The nature of the active processes concerned in absorption and secretion is still unknown; they are in all likelihood essentially the same in both functions; and from the analogy to other physiological processes in which the cell expends energy it seems probable that the production of differences of electrical potential between the surface layers and the interior of the cell is a fundamentally important factor. The view has, in fact, been entertained that cataphoresis plays a part in absorption,<sup>22</sup> although the experimental basis for this view is still inadequate.

<sup>21</sup> Compare Conklin: *Journal of the Academy of Natural Sciences of Philadelphia*, 2d series, vol. 12, 1902, part 1; pp. 45 seq.

<sup>22</sup> For the first time by Engelmann: *Archiv für die gesammte Physiologie*, 1872, vol. 6, p. 97. Cf. also Waymouth Reid: *Journal of Physiology*, 1901, vol. 26, p. 436, and Höber: *Archiv für die gesammte Physiologie*, 1904, vol. 101, p. 607.

There is, however, the following possibility to be borne in mind; if the changes of potential between the exterior and the interior of the cell, e.g., in the action-current, are due to alterations in the electrical condition of the cell-surface consequent on increased ionic permeability of the boundary layer—as the membrane theory supposes,—it is demonstrable that steep potential-gradients will momentarily exist between the superficial and internal regions of the cell at times of sudden increase in permeability;<sup>23</sup> this condition may be an important or even main factor in the transport of material through the cell, either in absorption and secretion, or in mitosis. The phenomena of mitosis, indeed, appear particularly significant from a physiological standpoint because of their furnishing strong indication that potential-gradients between the interior and the exterior of cells do in fact exist at certain periods, which are presumably periods of increased permeability. The radiations accompanying the process have the disposition of the electrical lines of force; they may be distorted or displaced, when once formed, by mechanical or other means, but in general the resemblance is unmistakable. If this point of view can be substantiated for mitosis, the significance of electrical forces in absorption and secretion, and probably in metabolism generally, will appear in a new light, since in mitosis all of these processes are concerned.

The significance to be attached to alterations of membrane-permeability in mitosis is thus, according to the above considerations, of a twofold nature: (1) the facilitation of interchange across membranes, and (2) the production, between different regions of the cell, of electrical potential-differences which play a direct part in the transport of material, besides being responsible for the characteristic radiations and spindle-formation.

The view that the mitotic figure with its astral radiations and spindle fibers is the expression of electrical potential-differences between the superficial and internal regions of the cytoplasm, and between cytoplasmic and nuclear areas, is one which I have advocated for some time. The chief difficulty under which such a view has hitherto labored is that of accounting for the existence of

<sup>23</sup> Cf. my paper in *Biological Bulletin*, 1900, vol. 17, pp. 207, 208; also *American Journal of Physiology*, 1910, vol. 26, pp. 128, 129.

such potential-differences. Granting that they exist, the radiating and spindle-shaped disposition of the colloidal material in the cell is readily understood, for colloidal particles in an electrical field must be affected similarly to other polarizable particles, and dispose themselves end to end along the lines of force, analogously to iron filings in a magnetic field. I propose to show that on the assumptions (1) that each semi-permeable membrane in the cell (nuclear membrane and plasma-membrane) is the seat of a potential-difference due to unequal permeability to anions and cations, and (2) that this potential-difference depends on the semi-permeable condition of the membrane and diminishes or disappears with marked increase in ionic permeability, it is possible to account for the existence of electrical fields within the cell having the characteristics requisite to produce the observed effects. All that will be attempted in the following pages, in fact, is to apply the membrane-theory of bioelectric processes to the case of the dividing cell.

This theory, which originated in a suggestion of Ostwald<sup>24</sup> and was first applied in detail to the bioelectric phenomena by Bernstein,<sup>25</sup> who has been followed by Brünings, Höber, and others,<sup>26</sup> is briefly as follows. In general any membrane, differing in its permeability to ions of opposite sign, and interposed between two electrolytic solutions of dissimilar concentration (or composition), must be the seat of a potential-difference; i.e., the two surfaces will have different potentials due to separation of oppositely charged ions at the membrane, since the more penetrating ion will traverse the membrane more freely and impart its charge to the layer of solution in contact with the opposite face. Such a potential-difference will persist so long as the inequality of concentration and of permeability to the two sets of ions remains unchanged. Equalization of the concentration-difference, as by gradual diffusion, will abolish the potential-difference; alteration in the permeability of the membrane, so

<sup>24</sup> Ostwald: *Zeitschrift für physikalische Chemie*, 1890, vol. 6, p. 71.

<sup>25</sup> Bernstein: *Archiv für die gesammte Physiologie*, 1902, vol. 92, p. 521.

<sup>26</sup> Brünings: *Archiv für die gesammte Physiologie*, 1903, vol. 98, p. 241, and vol. 100, p. 367; Höber: *Archiv für die gesammte Physiologie*, 1904, vol. 101, p. 607, also *Physikalische Chemie der Zelle und der Gewebe*; further references here.

that both ions then pass with equal readiness, will have a similar effect;<sup>27</sup> such an effect will follow any marked general increase in the ionic permeability of the membrane, since the selective permeability to different ions will then tend to disappear. If we take a limiting case and suppose that the membrane is freely permeable to one ion of the electrolyte, e.g., the cation (hydrogen-ion) of an acid, and completely impermeable to the anion, the surface adjoining the solution with the lower H-ion concentration will be positive relatively to the other surface; if the concentrations be known, the potential-difference can be calculated

from Nernst's equation,<sup>28</sup>  $E = \frac{RT}{F} \frac{u-v}{u+v} \ln \frac{c_2}{c_1}$ , for the potential-difference between two adjoining unequally concentrated solutions of any electrolyte with ions of unequal velocity; in the present case, since  $v=0$ , the potential-difference will be equal to  $\frac{RT}{F} \ln \frac{c_2}{c_1}$ .

This formula is identical with that expressing the factors determining the potential-difference between any ion-liberating surface, e.g., a metallic plate, and the solution, e.g., of its own salt, in contact with it.<sup>29</sup> Such a membrane in fact acts essentially as an ion-liberating surface, freeing ions (H-ions) with a certain solution-tension; it may thus play the same part as one of the ion-liberating surfaces (usually surfaces of metallic plates) in a galvanic battery. The same theory thus applies to the conditions under which potential-differences arise in a system containing

<sup>27</sup> The potential-difference in this case will fall to that existing between adjoining solutions of the electrolyte unseparated by a membrane.

<sup>28</sup> In this formula  $E$  denotes the potential-difference in volts,  $R$  the gas constant,  $T$  the absolute temperature,  $F$  the Faraday constant, i.e., the number of coulombs of electricity carried by a gram ion (here assumed to be monovalent),  $u$  velocity of cation,  $v$  of anion,  $\ln$  natural logarithm,  $c_2$  ionic concentration of the stronger,  $c_1$  of the weaker solution. The interposition of a membrane impermeable to anions of course reduces their velocity to zero.

<sup>29</sup> In such a case  $c_2$  is the solution-tension with which the ions are liberated from the surface,  $c_1$  the concentration of the ions in the solution. For a polyvalent metal the formula is  $E = \frac{RT}{nF} \ln \frac{c_2}{c_1}$ ,  $n$  being the valence of the cation.

such membranes as to the conditions in galvanic batteries.<sup>30</sup> Bearing this principle in mind, we find that the electrical phenomena exhibited by living tissues, i.e., systems in which electrolyte-solutions are separated by semi-permeable membranes—including those of stimulated muscle and nerve and dividing cells—lose their enigmatical character; they are special cases of phenomena long known to physical science and for which an adequate theory, due in its essential features to Nernst, has existed for some time.

The indications that electrically polarized semi-permeable membranes play a fundamental part in vital processes are many and various. Cells are separated from their media by surfaces which are definitely semi-permeable. They also show a potential-difference against the medium, the demarcation-current potential, which has been repeatedly shown to undergo marked decrease when the surface-permeability increases, as at death or under the influence of cytolytic substances. Another proof of a surface-polarization, undergoing decrease with increase of permeability, is the well known fact that living cells are carried by an electrical current toward the anode, indicating that the cell-substance is negative, the adjacent water surface positive; when the cells die the rate of transport undergoes decided diminution.<sup>31</sup> Again, electrical stimulation is a matter of ionic polarization, which can only occur at surfaces difficultly and unequally permeable to

<sup>30</sup> The type of membrane investigated recently by Haber and Klemensiewicz (*Zeitschrift für physikalische Chemie*, 1909, vol. 67, p. 385) is one permeable only to the ions of water. Such membranes, e. g. thin layers of glass or benzol saturated with water, have properties that appear in many respects to answer more closely to the biological requirements. Changes in the acidity or alkalinity of one of the solutions separated by such a membrane, amounting to a few ten-thousandths normal, may, especially if both solutions are nearly neutral, produce changes of potential comparable to those observed in living tissues during stimulation. The effects of increased permeability would in such membranes be identical with those produced at membranes of the type imagined by Ostwald. The most striking peculiarity of these membranes is their sensitivity to changes in the reaction of the adjoining solutions in the neighborhood of the neutral point. The fact that protoplasm and its normal medium lymph are typically neutral acquires new significance from this point of view. The following considerations apply to either type of membrane.

<sup>31</sup> An observation communicated to me by my colleague at Woods Hole, Dr. F. H. Pike, of Columbia University. I have since confirmed this observation, using portions of *Spirogyra* filaments.

ions; this has been definitely and conclusively demonstrated by the work of Nernst, Lapicque, Lucas, and Hill;<sup>32</sup> this theory is confirmed by the fact that during the stimulated state, when the membranes undergo increase in permeability, stimulation becomes difficult or impossible (refractory period); i.e., increase in the permeability of the plasma-membranes beyond a certain degree makes electrical polarization and hence stimulation, impossible;<sup>33</sup> this, it may be pointed out, is the essential reason why 'dead' cells are non-irritable. The proofs that stimulation involves increase in permeability of the plasma-membrane are too numerous to detail here;<sup>34</sup> electrical changes, as long known, are an invariable accompaniment of stimulation. All of these facts, with many others, indicate that the electrical condition of the boundary membranes of cells, i.e., of the semi-permeable plasma-membranes, is a matter of fundamental importance to vital processes, and that this condition is variable and dependent on the degree of ionic permeability of the membranes.

I have recently brought these facts and considerations to bear on the problem of cell-division.<sup>35</sup> Since free cells, like egg cells, show the same osmotic properties as muscle-cells, it is fair to assume that they possess the same electrical properties. Investigation indicates that this is in fact the case.<sup>36</sup> On application of the Ostwald-Bernstein theory of the origin of the demarcation-current potential to the case of the dividing cell, the familiar

<sup>32</sup> Nernst: *Archiv für die gesammte Physiologie*, 1908, vol. 122, p. 275. Lapicque: Numerous papers in *Comptes rendus de la Société de Biologie* and *Archives de Physiologie normale et pathologique*; cf. especially the latter journal, 1908, vol. 10, p. 601. Lucas: various papers in *Journal of Physiology*; cf. vol. 36, 1907, p. 253; vol. 37, 1908, p. 459; vol. 39, 1909, p. 461; vol. 40, 1910, p. 225. Hill, *ibid.*, vol. 40, 1910, p. 190.

<sup>33</sup> Cf. my paper in the *American Journal of Physiology*, 1909, vol. 24, pp. 17, 18, for a brief discussion of this point. Tait: *Quarterly Journal of Experimental Physiology*, 1910, vol. 3, p. 221, has brought forward evidence indicating that the duration of the refractory period is identical with that of the action-current. Both phenomena, on the present theory, are manifestations of the same essential change, viz., increase in surface-permeability.

<sup>34</sup> I have reviewed this evidence in *American Journal of Physiology*, 1909, vol. 24, p. 14, and 1911, vol. 28, p. 197. Cf. also *Science*, 1909, vol. 30, N.S., p. 245.

<sup>35</sup> Cf. *Biological Bulletin*, 1909, vol. 17, p. 188. *American Journal of Physiology*, 1910, vol. 26, p. 106.

<sup>36</sup> Cf. I. H. Hyde: *Amer. Journ. Physiol.*, 1904, vol. 11, p. 52.

phenomena of the production of a system of cytoplasmic radiations and spindle-fibers appear in a new light. The arrangement of the colloidal material in dividing cells irresistibly suggests the figures due to polarization of suspended particles in electrical or magnetic fields, and this comparison has naturally been made by many. The hypothesis that electrical forces are actually concerned in these phenomena has, however, been accepted by few and with reservations, and alternative attempts at explanation have on the whole received more credence among biologists. As I have already remarked, the difficulty of accounting for the existence of potential-differences between different regions of the cells has been the chief obstacle to the acceptance of such views. This difficulty, since the rise of the ionic theory and the recognition of the part played by ion-liberating surfaces in galvanic cells, has, I believe, ceased to exist. It is only necessary to recognize that membranes unequally permeable to anions and cations may play a part essentially identical with that of the metallic surfaces in batteries.

Evidence from many sides indicates that such membranes exist.<sup>37</sup> Furthermore, their existence is a necessary deduction from the ionic theory, which ascribes different diffusion-rates, different solubilities, and different velocities and powers of penetration to the different ions arising from the dissociation of any electrolyte. I shall not therefore attempt further in the limited space at my disposal to justify the assumption that such membranes exist; this would be tantamount to justifying the ionic theory, which, in spite of certain apparent inadequacies which have been made the basis of sometimes violent attack, has today a stronger position than ever. Its fruitfulness, the criterion of value in any theory, is attested by the innumerable successful researches which have

<sup>37</sup> Cf. Ostwald: *loc. cit.*; Tammann: *Zeitschrift für physikalische Chemie*, 1892, vol. 10, p. 255; Walden: *ibid.*, p. 699; Bein: *ibid.*, 1899, vol. 28, p. 439; Brünings: *Archiv. für die gesammte Physiologie*, 1903, vol. 100, p. 367; Schreiber: *Zeitschrift für physikalische Chemie*, 1899, vol. 28, p. 79; Springmann: *Annalen der Physik*, 1896, vol. 51, p. 140. The phenomena of electrical endosmose also belong here in part.

been based upon it.<sup>38</sup> Its application to the phenomena exhibited by surfaces and by matter in the colloidal state has been especially illuminating.<sup>39</sup> I shall assume, as in my former papers, that the plasma-membrane of any typical resting cell, like the egg-cell, is the seat of a potential-difference which is a function of its general impermeability to dissolved substances, including the majority of the ions normally present in protoplasm and its surroundings; it is further assumed that hydrogen-ions,—present in low concentration in protoplasm in consequence of the dissociation of carbonic and other weak acids produced in metabolism,—having high velocity and penetrative power, can freely traverse the plasma-membrane. Whenever, therefore, as normally, the H-ion concentration is greater within the cell than in its medium, the membrane will exhibit an electrical polarization with outer surface positive. The chief polarizing electrolyte on this hypothesis is thus simply carbonic acid. Hydrogen-ions penetrate the membrane and enter the adjacent medium; the corresponding anions, being blocked in their diffusion by the membrane, are left behind; a typical electrical double layer is thus formed. The protoplasm, like (e.g.) a zinc plate dipped in water, assumes a negative charge; there is a potential-difference across the surface, which, judging from the conditions in muscle, has an approximate value of 0.1 to 0.2 volt.<sup>40</sup>

<sup>38</sup> Even yet one hears doubts expressed among biologists as to the validity of the ionic theory. A good discussion of this topic is to be found in the paper by G. H. Lewis: "The use and abuse of the Ionic Theory," *Zeitschrift für physikalische Chemie*, 1910, vol. 70, p. 212.

<sup>39</sup> For the general subject of the relations of ions to surfaces and to matter in the colloidal state, cf. Michaëlis: *Dynamik der Oberflächen*, Dresden, 1909, and particularly Freundlich: *Kapillarchemie*. Leipzig, 1909. This treatise is a treasure-house of facts and principles of importance to biologists.

<sup>40</sup> I have met several times with the objection that carbonic acid is too weak to account for the very considerable potential-difference (*ca.* 0.1 volt) which, judging from the demarcation current of muscle, appears to exist between the outer surface and the interior of the cell. I do not see the force of such objections. The potential-difference is a function of *relative* concentrations on opposite sides of the demarcation surface, not of absolute concentrations. The quantity of ions actually liberated from a surface which shows a high potential-difference from its surroundings may be infinitesimal; e.g., take the case of a metallic plate like zinc in contact with a normal solution of its salt; the quantity of metal passing into solution is

In a resting cell like the unfertilized egg there are *two* semi-permeable membranes, the plasma-membrane bounding the entire cell and the nuclear membrane bounding the inner surface of the cytoplasm. The cytoplasm between these surfaces is typically homogeneous<sup>41</sup> and may hence be regarded as freely permeable to diffusible crystalloid substances, including ions. This is indicated by the fact that diffusible coloring substances, like the pigment of *Arbacia* eggs, tend to become uniformly distributed throughout the cytoplasm, but meet with barriers at nuclear and plasma membranes; Höber has recently adduced physico-chemical evidence indicating that ions are free to diffuse within the cytoplasm, though they encounter marked resistance at the plasma-membrane.<sup>42</sup> The semi-permeability of both nuclear and plasma membranes is evidenced by the difference between the inorganic salt-content of the cytoplasm on the one side and of both external medium and nucleus on the other. Numerous other proofs for the essential semi-permeability of the plasma-membrane are well known; Macallum's researches constitute the best proof for a similar condition in the nuclear membrane.<sup>43</sup> The

inappreciable; yet the P. D. is 0.51 volt. Particles of suspended quartz show a P.D. against the water of 0.044 volt. Cf. Freundlich: *Kapillarchemie*, p. 234.

It should also be noted that if the protoplasm serves in any way as a source of hydrogen-ions there will be a potential-difference at the surface even though the actual H-ion concentration within the protoplasm is extremely low. To account for the P.D. at the surface of the zinc plate it is not necessary to assume that Zn-ions exist as such in the metal; all that is required is that the zinc which passes beyond the surface into the solution should be ionized. There is thus no necessary discrepancy between the present hypothesis and the view which regards protoplasm as practically neutral in reaction. This, however, is demonstrably not always the case; contracting muscle (e.g.) may be distinctly acid to litmus, indicating a H-ion concentration exceeding  $10^{-5}n$ .

<sup>41</sup> This statement relates particularly to cells about to divide by mitosis. Such cells typically lack 'differentiation,' i.e., the colloidal as well as the crystalloidal material usually shows a uniform distribution. Egg-cells with their stored masses of inert food material or yolk are frequent exceptions.

<sup>42</sup> Höber: International Physiological Congress, September 1910; *Archiv für die gesamte Physiologie*, 1910, vol. 133, p. 237.

<sup>43</sup> Cf. Macallum: *Ergebnisse der Physiologie*, 1908, vol. 7, p. 552. Hamburger finds the nuclei of intestinal and tracheal epithelial cells decidedly less permeable to NaCl than the cell-bodies of the same cells; nuclei of other epithelia (bladder and oesophagus) are also impermeable to NaCl. Cf. *Osmotischer Druck und Ionenlehre*, vol. 3, pp. 8 seq. Römkes finds the nuclei of liver cells similarly impermeable (cf. *Biochemische Zeitschrift*, 1908, vol. 14, p. 254).

cytoplasm during rest, may therefore be regarded as an essentially homogeneous phase bounded externally and internally by a semi-permeable surface.

What will be the electrical conditions in such a system? As already explained, each surface is to be regarded as permeable to H-ions, hence as corresponding to an ion-liberating surface, like that of a metallic plate immersed in an ionizing solvent like water. The conditions will hence be essentially as follows: Imagine a hollow zinc sphere immersed in a solution of a zinc salt and containing in its interior a second solution of zinc salt (fig. 1). The

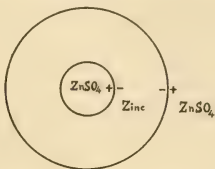


Fig. 1. Representing in section a hollow zinc sphere immersed in zinc sulphate solution and containing the same solution in its interior. The orientation of the double layers is shown. The metal is uniformly negative, the adjoining solution positive. The analogy to the conditions in the resting cell is obvious,—i. e., there are two concentric electrically polarized surfaces separated by a homogeneous conducting medium.

two surfaces correspond to the two semi-permeable surfaces in the cell; at each surface zinc ions will tend to pass into the solution with a pressure depending on the solution-tension of the zinc and on the concentration of zinc ions in the adjoining solution. There will thus be a potential difference at each surface calculable

from the formula:  $E = \frac{RT}{2F} \ln \frac{P_2}{P_1}$ ; the only possible condition of

equilibrium however will be that these two potential differences should be equal, since if one surface of the zinc be at a higher potential than the other, electricity will pass through the freely conducting metal to equalize the potentials.<sup>44</sup> The potential-

<sup>44</sup> The case is obviously different from that of a concentration-cell in which two metallic plates are immersed in unequally concentrated solutions of a salt of the metal, and the two solutions freely communicate. In the above system the two surfaces are in metallic connection *but the solutions are separated by an impermeable partition*. If the partition were rendered permeable to ions, e.g., by boring a hole through the sphere, thus placing the two solutions in communication, a typi-

difference at each surface will be the geometrical mean of that which either surface would show if the metal were in contact with only the one solution.<sup>45</sup> The metal will necessarily be isoelectric, i.e., of the same potential in all its parts (outside of the double layer itself); this potential will be negative by a certain value (equal to 0.51 volts for zinc in contact with a normal solution of zinc sulphate) in relation to both solutions in contact with the metal. This corollary is especially important from the present point of view, because it means that the central enclosed solution is positive in relation to the metal enclosing it.

Now the cell—considered from the electrochemical point of view as a system with two concentric semi-permeable membranes bounding a solution which in virtue of its slow oxidations is continually freeing carbonic and other acids and hence H-ions—must exhibit conditions essentially similar to the above. The seat of these oxidations is the cytoplasm. The region enclosed by the nuclear membrane must thus represent a region of higher potential than the adjacent cytoplasm, i.e., is positive relatively to the latter;<sup>46</sup> the same is true of the solution in contact with the surface of the cell. The persistence of such conditions depends on the semi-permeable character of the limiting layers or mem-

cal concentration-cell would be the result, and zinc ions would be deposited from the stronger solution and pass into the weaker while anions simultaneously by diffused from the more to the less concentrated solution until the two were equalized. Impermeability to anions is the essential characteristic of the space separating the two solutions in the above system as also of the space bounded by the semi-permeable membranes in the cell.

<sup>45</sup> The grounds for this conclusion will be found in Michaëlis' treatment of the case of a solid substance in contact with its saturated solution; this case is analogous to the above in principle. Cf. *Dynamik der Oberflächen*, p. 57.

<sup>46</sup> It is evident that this view assumes that the H-ion concentration within the nuclear membrane is less than that outside, i.e., in the cytoplasm. Such a view implies that the oxidative metabolism in the resting cell—and hence the production of carbonic and other acids yielding the H-ions—is essentially confined to the cytoplasm. The conditions in active tissues like muscle support this conception; in fact, the characteristic activities of cells are in general cytoplasmic activities; nuclei are relatively uniform in their characters. Hence the above assumption appears to be in accordance with the general facts of physiology. The question is difficult to decide by direct experiment, though possibly the use of indicators capable of penetrating both nucleus and cytoplasm without injurious action might yield valuable results. It should be borne in mind that oxygen, in order to reach the nucleus, must penetrate a layer of cytoplasm containing reducing substances.

branes, i.e., their essential impermeability to anions. Increase in the ionic permeability of the membranes must thus alter the electrical conditions in such a system, by altering the potential-difference across the surfaces concerned. The effect of such a change would be essentially the same as if in the above metallic model the solution-tension of the metal were to change.

One difference between the two analogous systems thus compared must here be emphasized, since upon it an essential part of the following physico-chemical interpretation of certain features of mitosis is based. In the metal inequalities of potential are instantly equalized. Alteration of the potential-difference at the surface, as by changing the concentration of the adjoining solution, must produce simultaneously an alteration of the potential throughout the whole metal, since electricity in metallic conductors moves with a velocity which, in relation to the distances involved in the case under consideration, is practically infinite. It is otherwise with a system consisting of an electrolyte solution bounded by membranes, like the cell. In this case an alteration of the surface potential-difference does not involve immediate alteration of the potential in the interior of the solution at a distance from the membranes, since electricity is conveyed in such a system only by the slowly moving ions;<sup>47</sup> hence an appreciable time must elapse before the solution is again isoelectric; during this period there will be a potential-difference between the surface-layers and the interior; the potential-gradient may have a very considerable slope, depending on the original potential-difference across the surface and on the distance between the membranes. If, as seems probable, ionic movement be slower in protoplasm than in simple aqueous solution this potential-difference may persist for some time; i.e., there will be an electric field within the cell during this interval.<sup>48</sup> To take a simple case: let fig. 2 represent

<sup>47</sup> The rate of ionic travel in protoplasm is of course unknown. It seems likely that it is slower than in simple aqueous solution because of the viscosity of the medium and the presence of colloids which adsorb or bind the ions and so limit their mobility. Cf. footnote 49.

<sup>48</sup> It should be remembered that there is direct experimental evidence that potential-differences, arising essentially in the manner described, i.e., by changes in the electrical polarization of a membrane separating two solutions, may be of considerable value and may persist for some time. I refer to the so-called polarization currents obtained from a membrane, through which an electrical current

a cell of the dimensions of an Arbacia egg (0.072 mm.); the nucleus is omitted from consideration to bring the conditions to their greatest possible simplicity. If the original surface potential-difference in the resting cell *A* be placed at the probable value of 0.1 volt, and it be assumed to fall suddenly to 0.05 volt as a result of increased ionic permeability (fig. 2, *B*), there will temporarily exist a potential-difference of 0.05 volt between the surface-layer and the interior of the cell; the gradient between the center and the periphery, a distance of 0.036 mm., will be *ca.* 14 volts

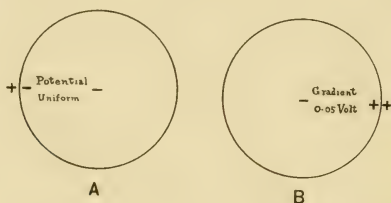


Fig. 2. *A* illustrates the conditions during the resting state. The plasma membrane is the seat of a potential-difference of *ca.* 0.1 volt with outer surface positive. The cytoplasm, within the double layer, is isoelectric, *i.e.*, the potential is uniform throughout. In *B* the membrane is supposed to have undergone decided and sudden increase in ionic permeability so that the surface polarization is diminished by 0.05 volt. Ions are then free to travel to equalize the potential-difference, and a gradient exists at first between superficial and central regions as represented. The fall of potential over this distance 0.036 mm. will at first be 0.05 volt, *i.e.*, *ca.* 14 volts per centimeter.

per centimeter. If we take the nuclear membrane also into consideration and assume an equal increase in permeability to occur simultaneously in both, the consequences will be essentially the same except that *two* oppositely oriented electrical fields will arise temporarily in the space between the membranes. It is to be noted that the regions most remote from the membranes will retain their original negativity longest. There will thus be a region of greatest negativity intermediate between the two membranes; from this region the potential will rise on either side toward

has been passing, for a certain time after the polarizing current has been broken. The same phenomenon, as is well known, is shown by living tissues, as muscle or nerve; dead tissues show it to a relatively slight degree.

the region adjoining the membranes where it will be highest. This condition can of course be only temporary since ions are free to diffuse through the cytoplasm. It is supposed, on the present theory, that it persists for a sufficient length of time to produce well marked effects.<sup>49</sup>

We are now in a position to apply the above principles in interpretation of the radiations and spindle-figure of dividing-cells. I shall consider only the most general and constant phenomena of mitosis, neglecting individual variations, and shall offer a physico-chemical-analysis of the conditions at a time when the mitotic figure is fully formed (metaphase). The radiations at this time centre toward two definite areas one on either side of the nucleus. Of those which immediately adjoin the nuclear region, two sets are ordinarily distinguishable, (1) the spindle-fibers which show a definite curved course, with concavity toward the cell-axis, similar to the lines of force between opposite electric or magnetic poles, and (2) the mantle-fibers which have a more external position, show a straighter course, and tend to diverge. The remaining radiations spread out from each astral centre in all directions toward the periphery of that half of the cell. If we regard the fibers as indicating with a fair degree of accuracy the direction of the electrical lines of force, we see here distinct evidence of the existence in each half of the cell of *two* oppositely oriented electrical fields. For reasons that will be apparent shortly, the peripheral regions of the cytoplasm are to be regarded as positive rela-

<sup>49</sup> I may cite here two quite independent investigations indicating that the rate of ionic movement in protoplasm may be much less than in ordinary solution. Girard has found that the diffusion-rate of electrolytes through membranes which are the seat of an electrical polarization is much slower than through the same membranes in the unpolarized condition. If this be a general rule, it would apply to the case of ions moving along potential-gradients in the cell. Cf. Girard: *Archives de physiologie normale et pathologique*, 1910, vol. 12, p. 471. Again, Keith Lucas concludes, from the differences in the excitation rate of various excitable tissues, that the ions concerned in the polarization resulting from electrical stimulation must move at vastly different rates in the different cases. The facts indicate that the ionic movement is 4000 times as rapid in a highly excitable tissue like the 'substance  $\beta$ ' of the frog's sartorius, as in the ventricular muscle of the same animal. If such a range of ionic velocities exists in different tissues, it is clear that in some the movement must be extremely slow. Possibly this condition is distinctive for dividing cells, which show an even slower rate of response than ventricular muscle. Cf. *Journal of Physiology*, 1910, vol. 40, p. 224.

tively to the regions toward which the radiations converge; the nuclear region is positive also. The lines of force in closest proximity to the nucleus are thus curved sharply toward the latter and in their aggregate show a spindle-form. Others on the nuclear side show less departure from the straight radiating course of the great majority; a certain number, intermediate in position are less strongly developed and show a less well defined course, peculiarities probably due to their being under the influence of both fields which partly counteract each other's action.

As to the origin of these two fields,—they arise, on the present hypothesis, in consequence of simultaneous and similar changes in the electrical polarization of the two boundary surfaces of the cytoplasm, due to alterations in the ionic permeability of the membranes. Let us assume the primary change to be a rapid and decided increase in the ionic permeability of the plasma-membrane.<sup>50</sup> This increase of permeability is assumed to be not uniform and simultaneous over the entire surface of the cell but to be most marked and most rapid over two extensive areas (e.g., between *A* and *B*, *C* and *D*, fig. 3) at the opposite sides of the cell adjoining the polar axis. It seems necessary to assume this definite localization of the areas of markedly increased permeability because of the characteristic and symmetrical bipolarity of the mitotic figure. If membranes are concerned in the process, a corresponding symmetrical and bipolar alteration of these structures must be assumed. There is at present no definite and independent evidence that this is the case; but the assumption seems justifiable since it involves merely the extension of the

<sup>50</sup> The question as to whether under normal conditions the nuclear or the plasma membrane is the first to undergo increase of permeability is probably to be answered differently in the different cases. In the majority of dividing cells the radiations appear to originate near the surface of the nucleus, typically at points where the nuclear membrane begins to break down, i.e., where its permeability first undergoes decided increase. This indicates that the primary change is frequently, perhaps usually, at the nuclear membrane. On the other hand, in artificial parthenogenesis or normal fertilization the primary action is on the plasma-membrane. The essential principle is that alteration of the potential-difference at the one membrane involves a similar alteration at the other. The case is analogous to the stimulation of one muscle or nerve by the action-current of another—stimulation signifying increase in the permeability of the limiting membranes.

general biological conception of an essentially bipolar organization in dividing cells so as to include the physico-chemical properties of the plasma-membrane. The increase in permeability must be sufficiently rapid and marked to produce a considerable fall in the potential-difference across the surface. On account of the interdependence of the two potential-differences at nuclear and plasma-membranes, a similar fall of potential must occur at the nuclear membrane. The latter change produces an increase in permeability<sup>51</sup> and is possibly responsible for the

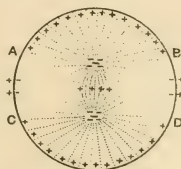


Fig. 3 In this figure the probable position and orientation of the gradients at the metaphase stage are indicated. The general disposition of the radiations is like that seen in the living sea-urchin egg. The + and - signs indicate the regions of highest and lowest potential in the several fields. The dotted lines represent electrical lines of force, corresponding to the paths of the diffusing ions. Several negative signs are crowded into the centers to indicate that the current density would be greatest there. The membrane over the equatorial region is supposed as yet to have undergone little or no depolarization.

dissolution of the membrane which shortly follows. The conditions are then essentially as represented in fig. 3.

The peripheral layer of cytoplasm and the central nuclear area are, for a time, positive, relatively to the adjoining regions. In each hemisphere a potential-gradient will exist between the positive areas adjoining the two depolarized membranes and the region midway between the two which retains its original nega-

<sup>51</sup> The facts of electrical stimulation, particularly Pflüger's law of cathodal stimulation on the make and anodal on break of the current, indicate that the permeability of the plasma membrane *depends* on its condition of electrical polarization, and that depolarization, whether partial or complete, involves an increase in permeability. The stimulating action of the electrical current depends on this: cf. my paper on the general conditions of stimulation in the *American Journal of Physiology*, 1908, vol. 22, pp. 77 to 80. Recently Girard has shown that electrically polarized membranes are in fact much less permeable to electrolytes than the same membranes when unpolarized. (See note 49.)

tivity the longest. The time during which this gradient will persist will be that required for the diffusion of ions to equalize the potentials. This diffusion is probably slow in the viscous protoplasm filled with colloidal particles, so that the gradients may persist for a considerable period. The space-relations of the regions of highest and lowest potentials would be approximately as represented; the region most remote from the two positive central and peripheral areas will naturally remain negative relatively to these areas for the longest period; this region will be intermediate in position in each half of the cell. Each hemisphere is thus the site of two oppositely oriented electrical fields. The potential-gradient of these fields, as the above considerations show, may at their first appearance be many volts per centimeter; their duration is problematical, but it seems probable that they would have marked effect, even if acting for only a few seconds.

The influence on the colloidal particles is apparently to produce electrical polarization analogous to that of most indifferent particles in strong electrical fields; the particles assume positions along the lines of force and apparently in many cases fuse to form fibrils. These fibrils once formed may persist, in accordance with their colloidal nature, for some time after the originating conditions have disappeared.<sup>32</sup> It should be noted that the definite character and sharp curvature of the rays connecting the astral and nuclear areas receive consistent explanation on the present theory, since the nuclear area is positive and the astral

<sup>32</sup> It should perhaps be emphasized that colloidal fibrils once formed may outlast the disappearance of the conditions to which they owed their origin. The essential process in their formation is the fusion of colloidal particles to form larger aggregates, as in coagulation. Now all degrees in the reversibility of the coagulation-process in protein solutions are known; some coagula (e.g., those produced by alkali or alkali-earth neutral salts) are readily, others difficultly reversible (heavy metal coagula). The fibrils formed in mitosis are often remarkably persistent (*Zwischenkörper*, etc.). It is clear then that once having been formed they may undergo curvature, displacement, or crossing, and the fact that they often do so under normal or experimental conditions (cf., e.g., Morgan: *Proceedings of the Society for Experimental Biology*, 1910, vol. 7, p. 132) in no way invalidates the theory that their *formation* is due to electrical conditions like those imagined above.

centers negative.<sup>53</sup> The chromosomes in the nuclear area, being negatively charged bodies, are influenced by the electrostatic forces existing in the field and spread out laterally in a symmetrical position between the adjoining negative areas. The lines of force, indicated by the radiations, converge in each hemisphere toward the intermediate regions of greatest negativity; the latter evidently correspond to the more or less definitely circumscribed astral centers.<sup>54</sup>

During this period ions must be regarded as slowly diffusing along the gradients in such a manner as to equalize the potentials. Just as the region midway between the two most positive areas (superficies and nuclear region) retains its original negativity longest, so the region midway between the two most negative areas (the astral centers) retains its original positivity longest. That is, the gradient of decreasing negativity or increasing positivity is from the astral centers toward the periphery and toward the original nuclear area. *Within* the nuclear area a region symmetrically situated with reference to the two astral centers will thus, so long as the potentials remain unequalized, be the region of highest potential or greatest positivity. The position of this region will coincide with a plane equidistant from and perpendicular to the astral centers, and to this plane negative particles will tend to be drawn; hence the gathering of the chromosomes in this 'equatorial plate' position.

<sup>53</sup>For the sake of definiteness I express myself here as if the negative and positive areas were sharply circumscribed; this of course cannot be the case where ions are free to diffuse; it is with more or less steep *gradients* that we are dealing. The astral centers, under the above conditions, represent merely the regions of greatest negativity; the plane midway between the two, the region of the greatest positivity; the potential changes continuously and probably quite uniformly—judging from the usual disposition of the fibrils—between these two regions. The conditions are essentially like those in the solution between the plates of a battery with closed external circuit, though the gradient in the cell is much steeper.

<sup>54</sup>The degree of development of the radiations will depend on the steepness of the gradient, on the time during which it persists, and on the density of the current-lines. This is probably why the spindle-fibers are the most constant, definite, and persistent of all; it also suggests the reason why radiations do not normally extend from the nuclear region to the equator of the cell. Possibly the neighborhood of the other oppositely oriented fields also interferes with the formation of such radiations.

I do not propose to elaborate the above theory further at present. The foregoing analysis, whatever its defects in detail, indicates, I believe, that by taking account of the changes of potential resulting from alterations in the permeability of electrically polarized membranes, certain characteristic phenomena of mitosis are susceptible of consistent physico-chemical explanation. The distinctive appearances presented by the process appear to depend essentially on the following conditions: that two concentric semi-permeable membranes, enclosing between them cytoplasm with its characteristic oxidative metabolism, undergo simultaneously, in certain definite regions, alterations of permeability with accompanying changes in the potential-differences between the regions which they separate. Membranes of varying permeability thus play a fundamental rôle in cell-division, as in the processes of stimulation, absorption, secretion, and conduction of stimuli.

It is evident that such a system as the dividing cell presents many difficult physico-chemical problems. The situation is complicated by the chemical changes involved, some of which, including the energy-yielding oxidations, must be influenced by the changes in electrical potential at the surfaces. The membranes, in fact, are to be regarded as electrodes of changing potential; and by acting as such, as well as by altering the conditions of interchange, they must influence the course of the chemical reactions. The simple inorganic model imagined above is probably similar, in the purely electrochemical aspect, to the normal nucleated cell, while complications of a purely chemical nature are absent. It may thus serve to throw light on those physical features of the mitotic process which are dependent on changes of electrical potential at the surfaces. The nature of the metabolic processes in cell-division remains, however, as the essential problem, and it is probable that the facts required for its adequate solution are still largely unascertained. It is possible that study of the relations between alterations in membrane-permeability and chemical changes in substances adjacent to the membranes will throw light on the nature and conditions of metabolic processes, not only in dividing cells but in cells in general.

# THE SPERMATOGENESIS OF AN HEMIPTERON, EUSCHISTUS

THOS. H. MONTGOMERY, JR.

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147 FIGURES—FIVE PLATES

## CONTENTS

Introduction .....	732
The autosomes (ordinary chromosomes).....	733
A Observations .....	733
B Discussion: continuity, conjugation and reduction .....	747
The allosomes (modified chromosomes).....	755
A The idiochromosomes and the discharge from the nucleus of the sper- matozoon .....	755
B The minute chromosomes .....	760
C Discussion .....	761
The plasmosomes .....	762
A Observations .....	762
B Discussion: genesis and kinds of nucleoli .....	764
The chromatoid corpuscles.....	767
Cell axes, centrioles, flagella.....	768
Cytoplasmic structures .....	772
A Mitosome and cell plate.....	772
B The idiozome .....	773
C The spheres .....	774
D The mitochondria .....	776
E Discussion .....	782
Preformation and epigenesis in the germinal cycle, and segregation of the germ cells in ontogeny.....	790
Literature cited.....	792

## INTRODUCTION

This study was undertaken mainly to determine two questions that at present are of great theoretical importance, the mode of the conjugation of the chromosomes, and the origin and distribution of the mitochondria: but I have found it necessary to investigate most of the other phenomena of the spermatogenesis at the same time, consequently I have been led to describe the entire spermatogenesis up to the ripe spermatozoon, thus completing the investigation of this species begun by me in 1898.<sup>1</sup>

Considerable time was given to the study of living cells, in which the testis was teased and placed in Ringer's solution. It was a great surprise to me how much could be seen in this state: plasmosomes distinguished from allosomes during the growth period, the mitochondria very distinct, in the maturation mitoses the centrioles and mantle fibers as clear as upon the best stained preparations and the chromosomes visible, though not so clear, and all the phenomena of the histogenesis of the sperm quite as distinct as upon any stained preparations. By this *intra vitam* study a pseudopodium of the spermatid was found that could not be seen upon sections, and the stages in the shrinkage of the spermatid nucleus be seen with the greatest clearness. This has convinced me that the fixed preparations we have all been studying present a very close picture of living conditions, provided a good fixative like Flemming's stronger fluid is employed. Despite what microchemists may conclude, we have been working with images that are very close to the living, and that may well be a source of great consolation. Indeed, the argument that cytologists have for the most part studied only precipitates and coagulates need no longer be advanced to us, when structures seen in life so closely approximate those of fixed and stained sections.

More than forty testes were sectioned after fixation by a variety of methods, though those fixed by Flemming's and Hermann's

<sup>1</sup> This is the species I have called *E. variolarius* Pal. Beauv. and a discussion of the identification is given in my paper 1910a.

mixtures were the ones mainly studied. Two principal stains were employed, the iron haematoxylin of Heidenhain and the safranin-gentian violet method of Hermann—the latter unequaled for many purposes. For the mitochondria in particular a number of slides were stained with Meves' modification of Benda's method, but no precise differentiation was reached, other bodies always staining at the same time as the mitochondria. My clearest and most satisfactory stains of the mitochondria were reached with the iron haematoxylin method, in which it is best to use rather thick sections and to destain only slightly.

The greater number of the drawings were made from two particular testes, nos. 265 and 282, the former because it showed the mitochondria most distinctly, the latter because it was the best for the study of the chromosomes and the spheres. There is so much variation in small details between cells of correspondent stages from different testes that it seemed best to describe the phenomena as they occur mainly in one individual. Further, of the six longitudinal follicles of the testis, the cells of only follicles 4, 5 and 6 were studied, for these cells are of approximately the same size; the large spermatocytes of follicles 1 and 3 present certain peculiarities that it seemed best to leave to a later special study.<sup>2</sup>

The final writing up of this paper had to be done under great stress of outside duties, so that in my discussion of the literature I may have made some important omissions.

## THE AUTOSOMES

### *A. Observations*

Under the term 'autosomes' are meant the chromosomes of the ordinary type, in distinction from the modified chromosomes or allosomes that differ from them in behavior; this terminology was introduced in an earlier paper of mine ('06).

<sup>2</sup> On the differences of the cells in the six follicles, see my paper, 1910a.

Spermatogonia may always be distinguished from spermatocytes of the earlier growth period by the following characteristics: (1) the spermatogonia are arranged in radial rosettes; (2) each of them possesses a mitosome in the rest stage; (3) their plasmosomes do not lie against the nuclear membrane; (4) their cytoplasmic mass is relatively larger; (5) they never possess filiform mitochondria. These differences are mentioned in order to forestall any objections that might be raised to our seriation of stages, such as confusing a telophase of a penultimate spermatogonium with that of an ultimate one, an important point, for telophases of ultimate spermatogonia are necessarily first spermatocytes. There is no possibility of confusing spermatogonia with any other cell generations than early spermatocytes, for the successive stages of the spermatogenesis follow each other along the length of each testicular follicle in fairly regular order.

In testes of adult individuals are found two generations of spermatogonia, which it will be convenient to call the 'penultimate' and the 'ultimate,' these terms being preferable to 'primary' and 'secondary' of most writers, for the reason that primary and secondary employed in the strict sense should refer to the first two generations of the germinal cycle. Whether there are three generations of them in adults was not positively ascertained. Fig. 1 represents a penultimate, and fig. 3 a group of ultimate, spermatogonia, and these figures give a fair example of the usual differences between the two. The penultimate spermatogonia are larger, usually shorter, and their number within a cyst (rosette) is only half that of the ultimate spermatogonia.

In resting cells of both spermatogonial generations a portion of the chromatin is always arranged on the nuclear membrane in the form of a chromatin plate, in the immediate vicinity of the idiozome (figs. 1 and 3). The local connection of these two structures is invariable, no matter what the position of the idiozome may be; this relation was overlooked in my paper of 1898.

In equatorial plates of the spermatogonia are to be counted fourteen chromosomes (fig. 2), twelve of which are autosomes as detailed particularly in my papers of 1906 and 1910. The very

smallest is the smaller idiochromosome (*d*). The autosome pairs present constant size and form differences, and at least five pairs (*A, a, B, b, C, c, E, e, F, f*) may be readily distinguished from the others. But of the three remaining chromosomes, contrary to my conclusions of 1906, I do not find it possible to decide which two are autosomes and which one is the larger idiochromosome, but that one of them is necessarily the larger idiochromosome we shall see is unquestionable. All of the twelve autosomes divide in every spermatogonial mitosis, for each first spermatocyte invariably comes to contain twelve, as detailed especially in my paper of 1910.

Fig. 4 exhibits an early telophase of an ultimate spermatogonium. The chromosomes are here not fused, as the figure might imply, but merely so closely apposed that they can be distinguished only with difficulty in a side view.

The daughter cells resulting from this division are the first spermatocytes, and these are shown in their earliest stage in fig. 5. In the upper of the two cells there drawn, fourteen chromosomes can be counted, and in the lower, thirteen; in the latter it is probably the smaller idiochromosome that is hidden from sight.

The next change is that the nucleus becomes larger while the autosomes grow more irregular in form, become less compact and swell in size, as shown in figs. 6 and 7, each of which exhibits fourteen chromosomes. A few delicate linin threads appear joining autosomes, but the connective fibers of figs. 4 and 5 (derived from the linin constituent of the chromosomes) have disappeared without a trace, and so have the granules of the cell plate. Then the autosomes begin to lengthen (figs. 8 and 9), showing sometimes amoeboid contours (figs. 10 and 11), and soon become linear (figs. 12 and 13). The precise succession of the stages of figs. 6 to 12 is difficult to determine, but without doubt there transpires a gradual lengthening of the chromosomes at this time without either splitting or conjugation. Figs. 13-15 represent succeeding stages of this lengthening of the autosomes, and there can be no doubt that the stage of fig. 12 passes

immediately into that of fig. 13, for both these cells lie within the same cyst.<sup>3</sup>

The cells of figs. 16 to 19 correspond approximately to the stage where, in most other objects, there is an intercalated rest period. But in *Euschistus* there appears to be no rest stage, but at all times chromosomal boundaries may be clearly distinguished. I have given particular attention to this point, for it is one of considerable importance with regard to the question of the continuity of the chromosomes.

Figs. 22 to 24 lead to the leptotene stage that is shown in figs. 25 to 28 (figs. 25 to 27 being cells from one cyst). A good criterion of this stage is the first indication of beginning conjugation of the still irregular idiochromosomes. There is at this period occasional indication of parallel grouping of some of the autosomes (e.g., in fig. 27), evidently the first step in the conjugation of the autosomes. In the leptotene condition there is certainly no continuous chromatin spirem developed, for free ends of certain autosomes may always be distinguished. Indeed, at no time in the history of the spermatocytes is there a continuous chromatin spirem produced. This stage is evidently of short duration for it is seldom found.

In nearly every stage, from the leptotene to the pachytene, some cells of nearly every cyst may exhibit the condition of synizis, closely massed grouping of the autosomes. That is, synizis of the autosomes is not limited to a particular period but extends over quite a series of stages, and at any one of the stages some cells may exhibit it while others do not. This may then be a

<sup>3</sup> Objection might be raised that, with the seriation of my stages, cell and nuclear size is not always conformable. But cells in the same cyst vary considerably in volume. Thus fig. 9 is one of the largest cells of its cyst, the others of which were smaller, and figs. 16-19 illustrate cells of very different volumes but all from one cyst. Size of cell or nucleus is then no good criterion of stage. The matter of a correct seriation of stages is so important and one is so liable to make mistakes concerning it, that I have seriated the stages not primarily by a study of the autosomes themselves, for that would be arguing in a circle, but by the seriation of other components, particularly the plasmosomes, the idiochromosomes, and the cytoplasmic differentiations. The reader will find, on examination of plates 1-3, that my seriation takes general count of the changes of all these structures.

condition of periodical or rhythmical occurrence, perhaps associated with periodical discharges of nuclear substance, the same cell passing through a series of such conditions; in no other way at least can the sporadic occurrence of synizesis be explained. For this is clearly a normal phenomenon and not an artefact due to faulty preservation of the nuclei, for it is seen after the use of all fixatives and only up to the pachytene stage. It is then associated with the time when the nuclear membrane is exceedingly delicate. Figs. 23 and 24 from one cyst, and figs 30 and 31 from another, illustrate the different appearance that cells of the same cyst may show. But even during the acme of such synizesis (figs. 24, 31, 33, 27, 40) there is no evidence that the autosomes become fused or in any way lose their identity, all the appearances indicate rather that they are then only very closely massed; for in thin sections of such masses the outlines of the individual autosomes may always be discerned.

The leptotene condition with its slender but still isolated autosomes (figs. 25 to 28) passes gradually over into the zygotene, leading to the condition where the autosomes lay themselves parallel in pairs (figs. 41, 42). It is difficult to be sure of the exact sequence of all the stages shown in figs. 29 to 42, yet there can be no doubt that the leptotene condition changes gradually into the zygotene. It will be recalled that no continuous chromatin spirem had been produced, therefore the bivalent autosomes cannot be formed by any transverse breaking of such a spirem. Already in the leptenema there may be some trace of a parallel juxtaposition of autosomes, and this would seem to have proceeded further in the stages of figs. 29 and 30. Then we reach the interesting conditions of figs. 32 to 35 and 38 to 42; in figs 32, 34, 42 all autosomes are shown in their entirety. In fig. 32 most of the autosomes show parallel arrangement, especially marked in the case of the pair that touch the plasmosome (*Pl*). Fig. 34 is especially important; it exhibits, in addition to the two idiochromosomes (*D*, *d*), precisely twelve autosomes, four of which compose two gemini; in other words, this nucleus shows eight univalent and two bivalent autosomes. This figure, made with the greatest care and redrawn on different occasions, estab-

lishes, together with the other evidence that is to follow, that we are dealing here with parallel conjugation and not with longitudinal splitting. For there being in fig. 34 just ten threads, two of which are double, and the spermatogonial number of autosomes being twelve, it necessarily follows that each of the double threads could have been produced only by juxtaposition of two single threads. In fig. 38 the pairing of certain autosomes is seen clearly. In fig. 42 where the entire nuclear content is shown, there are seen exactly six chromatin loops (excluding the pair of idiochromosomes, *D*, *d*), and each of these is double; these are then six bivalent elements, each produced by the lateral apposition of two univalents. In fig. 41 five entire double loops are drawn, the sixth being omitted so as not to obscure the picture. Thus it results that the six bivalent autosomes or gemini of figs. 41 and 42, and consequently of later stages, are produced by lateral conjugation of univalent autosomes; and the clear line that one sees along the axis of each such double thread cannot be a longitudinal split of a single univalent.

The stages delineated in figs. 34 to 42 have been arranged according to the stage of cytoplasmic differentiation, particularly the growth of the sphere. This cytoplasmic development does not keep exact step with the nuclear, for, e.g., the cell of fig. 39 contains exactly eleven autosome loops, therefore cannot contain more than one bivalent, and with regard to its nuclear components should precede fig. 34 that contains two bivalents.

The zygotene condition is closely followed by the pachytene (figs. 43 to 45), in none of which are all the gemini represented; this stage is found just before the sphere (*Sp*) has reached its greatest size. Here the autosomal loops are in one-half the normal number, and, for the most part, each of them appears solid and undivided. But in the greater number of cases there is to be seen on each geminus at least traces of a clear space which marks the original point of meeting of two univalents. Thus in *Euschistus* there is no valid evidence of any actual fusion of the two members of any pair.

It will be convenient to stop at this point to recount another process of the autosomes. In the earliest growth period (figs.

6 to 14) certain autosomes are regularly in contact with the nuclear membrane at the distal pole of the latter, where it touches the idiozome (*id*). This continues until the disappearance of the idiozome (figs. 47, 48), after which none of the autosomes maintain such a position. In earlier stages (figs. 6 to 12) there appear to be two autosomes invariably in this position, but later it would seem that the ends of more than two touch the nuclear membrane at the idiozome pole (figs. 14-20, 23, 25, 27-29, 33-43). Otherwise autosomes do not end upon the nuclear membrane except in connection with the growing plasmosome (to be described in another place). Whether these are particular autosomes, or whether it is a matter of chance which of the autosomes occupies this position, I have not been able to determine, but this is a regular phenomenon of every cell of these stages. The ends of such autosomes are coiled upon the nuclear membrane, making there a plate of chromatin and not a disc of separated granules as described by me in 1898. This 'chromatin plate,' as it may be called for convenience, is invariably at a particular pole, next the idiozome body, and probably has something to do with the genesis of the latter as will be shown later. This plate is brought to an end by the autosomes withdrawing from the nuclear membrane, and leaving no chromatin behind them. But I have seen no evidence that any chromatin is bodily eliminated from the nucleus at this point.

Following upon the pachytene (figs. 43 to 45) comes the strepsinema or diplotene stage, the earlier part of which about coincides with the maximum size of the sphere and its dissolution (figs. 46 to 57). The pachytene threads gradually unravel so that each geminus comes to appear distinctly double for the second time. The two components of each geminus then appear more or less spirally twisted around each other and their shortening is due to this twisting rather than to any linear contraction. As a rule the two components of a geminus begin to separate first in their middle portions, while their ends are still closely apposed (figs. 50, 54, 56). Three entire gemini are drawn in figs. 46, 47, 49, 54, 55, two entire ones in figs. 50, 52, and four in fig. 56. In fig. 48 five gemini are shown in their entirety, while the sixth, to avoid

complicating the drawing, is represented as a line simply to show its position and length. A comparison of fig. 48 with fig. 42 demonstrates that just after the pachytene condition the bivalents have the same number and form as just before it, another indication that their univalent components had not fused in the pachytene stage. In the earlier portion of the strepsinema all six bivalents may be readily distinguished and their boundaries accurately determined, provided one select nuclei where they are scattered and not massed and then draw their outlines patiently with the camera lucida. In most of the cells of this stage in order to show their details with the greatest distinctness only a few of the gemini have been drawn.

Generally the two components of a geminus appear of equal thickness and length, but occasionally there is to be noted a difference of their diameters as in the case of the right hand geminus of fig. 49.

Shortly after the time of disappearance of the sphere in the cytoplasm the autosomes become for a while grouped around the plasmosome, as exhibited in figs. 58, 60, 61-66, fig. 61 showing the most common appearance of autosomes converging towards the plasmosome. Most of the figures are incorrect in one respect, namely, in omitting to show how the gemini end upon the plasmosome, a relation difficult to determine because at this stage the plasmosome stains deeply, but they do not appear to penetrate into the plasmosome substance. The meaning of this phenomenon, which is a constant one for this period, will be discussed under the subject of the plasmosome.

The later history of the autosomes shows that the gemini widen out in such a manner that the two univalent components of each continue to be connected at one end while diverging at the other, the end result being, in the definitive gemini of the reduction division, every two univalents joined end to end. This process is effected gradually, it does not take place in all cells at the same time nor yet at the same time on all gemini of the same nucleus, and in its details shows considerable variation. Thus in figs. 50, 53, 56 the univalents are more widely divergent than in fig. 59, though the latter is a later stage of the cytoplasmic

structures; fig. 59 exhibits five entire gemini, and by a heavy line the position of the sixth. The two univalents of each geminus, as they begin to unravel, appear at first spirally twisted around each other (figs. 48–57). Then they begin to diverge at one end. The point of continuing contact of the two univalents of a geminus, or point of persisting conjugation, is marked by *x* in figs. 58, 60, 62.

No rest stage follows this condition, contrary to my first description, but the gemini immediately approach the nuclear membrane and the prophases proper of the first maturation mitosis are established.<sup>4</sup> Fig. 63 marks the beginning of the prophase, and later prophases are shown in figs. 64–86. The concurrent changes of the gemini are theoretically so important that they deserve to be described in detail. In fig. 63, which presents the greater portions of five gemini, the conjugation point of each geminus is marked by the letter *x*. Figs. 64 and 64a show five entire gemini of one nucleus, the sixth being omitted because it lay in an oblique position; this shows various stages of gemini, the one of fig. 64a illustrating particularly clearly how the univalents remain attached at one point and separate at the other. The conjugation points, marked by *x*, can be seen in some of the gemini of figs. 65, 66, the latter exhibiting all the gemini, but in both of these the elements lie so obliquely as to obscure the relation. As the prophase advances the process becomes clearer, for the autosomes gradually become shorter and more regular in form. Thus in fig. 67 two whole gemini are drawn, each of which clearly possesses a V-form; the same condition is shown somewhat less clearly in figs. 69 and 70; in figs. 71–84 also, the letter *x* denotes the conjugation point. Figs. 71 and 72 are from one cyst, the latter showing two gemini. Fig. 73 illustrates a part of a geminus on the right and a whole one on the left, the latter exhibiting clearly the mode of

<sup>4</sup> Stages like those of figs. 57–62 are in the large spermatocytes of follicles 1 and 3 very much more like rest stages, for in them the autosomes become more diffuse and their boundaries more difficult to determine; it was from a study of those large cells that I was led, in 1898, to conclude the occurrence of a rest stage at this period. It will be recalled that in the present paper the conditions in follicles 1 and 3 are disregarded.

junction of the univalents. Fig. 74 shows two entire gemini and fig. 75, of another cell of the same cyst, three entire ones. Figs. 76 to 80 show gemini from cells of one cyst, of which figs. 76 and 78 show each two gemini and the others each one. Fig. 81, of a stage when the gemini are more condensed, shows four whole gemini; fig. 82, a cell of the same cyst, two whole gemini; and fig. 83, still another cell of the same cyst, shows all the gemini. The last three figures, from cells of the same cyst, illustrate how rapidly these changes succeed each other. In figs. 84 and 85 all the chromosomes are shown, and in each of these cases there are six bivalent autosomes.<sup>5</sup>

By the stage of fig. 85 most of the gemini have become straightened out, though two still retain the angular form. In the late diakinesis of fig. 86, in which again all the chromosomes are drawn, all the autosome gemini have become straight with an annular constriction about the middle, the 'dumbbell shape' of my previous descriptions.

How are we to evaluate the definitive gemini of the first maturation mitosis, shown in figs. 87 to 89, and 92 to 94? Their history, as we have followed it, demonstrates that the twelve univalent autosomes were at first distinctly separate, they do not at any time produce a continuous spirem, they undergo a parallel conjugation, then in each geminus the two components begin to separate at one end while remaining attached at the other (this the persisting conjugation point). Thus two parallel autosomes change into a V-shaped geminus, and finally the two arms of the V open out into a straight line. Accordingly, each geminus of the first maturation division represents two univalents placed end to end, and the constriction around each geminus marks the point of persisting conjugation of the two univalents.

No other interpretation seems justified after a long and repeated study of the whole series of phenomena. And a degree of certainty can be reached because there is no rest stage at any period

<sup>5</sup> In fig. 84 one of these gemini has its two components, s, s, separated from each other. This is a frequent variation, and the history of such s-chromosomes I have detailed elsewhere ('10a).

of the spermatocytes, nor is a continuous spirem produced, and the univalent autosomes preserve their long axes throughout.

Chromosomes most difficult of interpretation are ring-shaped ones, and fortunately *Euschistus* has none such in the maturation mitoses. Gemini of such form are, however, frequent in the pro-phases as shown in figs. 69, 70, 74, 75 and 82 to 84, even though these later invariably straighten out into rod form. In earlier papers I have shown that such a ring is a geminus in which the middle part has widened out while its ends have remained closed; other gemini differ in having the univalents separated at one end while apposed at the other. The inception of a ring is very simple. From such a stage as that of figs. 49 to 55 where every two univalents are wound around each other, is attained the condition shown in figs. 56 and 64 where the univalents begin to separate along their middle portions. The latter are incipient rings and they may remain such until a late prophase, or may change into V-shaped gemini by breaking their contacts at one end. Neither rings nor Vs are produced by longitudinal splitting. It is quite possible that certain gemini may be regularly ring-shaped and other regularly V-shaped during the prophases. For, as shown in figs. 69, 74, 75, 82, and 83, the rings are usually of conspicuously large size, and usually if not invariably in the number of one to a nucleus, as if such a geminus always represented a particular large pair of autosomes and were not a mere variation.

It is now necessary to examine at what period the autosomes become longitudinally split. In the growth period through the pachytene stage there is no longitudinal splitting, for what I had previously ('01) interpreted as such, I now find to be the line of conjugation. This is proven sufficiently by the fact that the twelve autosomes of the spermatogonia are represented in these stages by six double rods. Frequently at certain points along a geminus the chromatin granules appear accurately paired (figs. 51, 52). But this does not appear until a rather advanced stage of the strepsinema and is by no means regular. The true longitudinal splitting of the univalent elements takes place at a much later stage than I had previously supposed. Occasionally in a very early prophase there is an indication of it, as in fig. 64a.

But this is unusual, for in the series of later prophases of figs. 67 to 78 no trace of it was noticed. It is when the autosomes have shortened and their surfaces become nearly smooth that this split is prominent for the first time. The gemini of figs. 79, 80 show it prominently but in each case upon only one univalent. It is fairly conspicuous along the larger geminus of fig. 82; in fig. 84 it is seen along two of the gemini; the reason why it is not seen upon all at this stage is probably because it is visible only when the flattened surface of a univalent is turned towards the observer. During the first maturation metaphase this split can always be seen when the gemini lie in the proper position and when they are sufficiently distended (figs. 86-88, 93-94).

During the strepsinema stage and the consequent prophases the nuclear linin threads increase in number, as shown in figs. 42 to 93, and there are good indications that they are outgrowths of the autosomes. In the later prophases these become replaced by chains of fine globules (figs. 78-85). The latter appear to emanate from the autosomes, as though they were droplets of fluid pressed out by these during their process of condensation; and it may be that such droplets pass out along the linin threads, for this would explain their catenulated arrangement. This appears to be the only act of intranuclear substance emission by the autosomes, for in earlier periods the karyolymph appears quite clear.

The history of the autosomes during the maturation mitoses has been so fully described by me before ('01, '06, '10) that it needs but brief mention here. The six bivalent autosomes become arranged in the equatorial plane of the first maturation spindle with their long axes parallel to the latter (figs. 87-89; 92-94). All the chromosomes are drawn in fig. 89. Their longitudinal splits are also in the line of the spindle, and on polar views of the equatorial plate (figs. 90, 91) this split is often evident as an indentation upon the autosome. The transverse constriction of each geminus, which is the point of persisting conjugation of two univalents, lies in the equator and the autosomes divide along it (figs. 94, 95). This first division is then reductional, and gives to each second spermatocyte six univalent autosomes. As the

daughter (univalent) autosomes separate (figs. 95-97, 99, 100), the longitudinal split becomes much more pronounced upon each, appearing first as a deep indentation at the point nearest the equator of the cell. On polar view of an anaphase each autosome shows a constriction which is the split. In the second mitosis (figs. 102, 103) that follows without any interkinesis, the univalent autosomes divide along the line of this constriction, hence equationally, so that each spermatid receives a half of each of the six univalent autosomes (figs. 105, 106). In the equator of the second maturation spindle the autosomes are arranged usually in a circle around the pair of idiochromosomes (fig. 101). In the anaphases represented in figs. 104 and 107 to 109 individual autosomes are not delineated but only the masses of them, for on lateral views it is difficult to make out their outlines; in fig. 110 five autosomes are shown in each daughter cell, the sixth in each being obscured by one of the others.

In the histogenesis of the spermatid the six autosomes early become irregularly massed in a circle around the idiochromosomes; in fig. 111 they happen to be so closely massed that their individual boundaries cannot be determined, yet there is no fusion of them. Fig. 112 shows the delimitation of the young daughter nucleus by the formation of its membrane (in the stages of figs. 110, 111 the chromosomes lie in a vacuole not yet bounded by a membrane), and the six autosomes in peripheral position around the central idiochromosome (*d*). From this time on the autosomes remain strictly peripheral, until they finally produce the hollow cylinder of chromatin that composes the major portion of the sperm head. In fig. 113 the nucleus has grown in volume, and the autosomes have also become larger and less dense. Figs. 114 to 117 illustrate succeeding stages, the nucleus attaining its maximum size and the autosomes becoming flattened against its membrane; fig. 117 shows four of the six autosomes, all that can be seen on profile, the remaining two being on opposite surfaces. The evidence is clear that the whole, or at least the major part, of the substance of the autosomes becomes peripheral, and that the larger bodies (*D*) that lie free within the nuclear cavity are derivatives of the idiochromosomes. By the stage of fig.

118 the autosomes have become so spread out that they compose a thin layer of chromatin covering the whole inner surface of the nuclear membrane except at the pole nearest the centriole (*c*). From now on boundaries of the autosomes can no longer be distinguished. This peripheral mantle of chromatin appears deeply stained when viewed in section, but pale in surface view. In the stages of figs. 119 to 126 there is no marked change in this layer of chromatin, beyond that the area of interruption opposite the centriole becomes reduced. Then the whole nucleus becomes smaller, with enlargement of the area of interruption of the chromatin (figs. 127-136); this phenomenon is associated with discharge of substances from the nucleus and will be described in the section on the idiochromosomes. It next begins to elongate (figs. 134-138). Later (figs. 139, 140) it becomes laterally compressed, the peripheral chromatin again extending nearer the centriole. Thus the chromatin cylinder lengthens and grows bilaterally symmetrical; fig. 141 exhibits it from its flattened surface, showing how the border next the perforatorium is more arched, while the opposite surface remains more flattened. Figs. 142 and 143 illustrate two immature sperm of a later stage from one cyst; the first is seen from its flattened surface while the other is viewed from its narrow edge; thus the sperm head has grown into the shape of a shallow, pointed spoon. At the same time it becomes spirally twisted to a slight extent. A later stage, seen from the flattened surface, is shown in fig. 144, and a cross section of a head of the same stage in fig. 145. The latter figure illustrates how the concavity of the head has become a deep groove and it is this longitudinal groove that might mislead one, viewing the head from the side, into supposing that there were an axial rod contained within the cylinder of chromatin. In this way the sperm head changes from the shape of a bent spoon to that of a narrow, pointed rod, with a deep groove on that surface directed away from the perforatorium. A later condition is drawn in fig. 146, and a mature sperm head from the distal end of the testis in fig. 147. Seen from its more flattened side the mature head shows as a thin hollow cylinder of chromatin containing a distinct cavity filled with nuclear sap, but seen from its

narrower edge it more frequently seems to be a solid mass of chromatin. The nucleus thus remains vesicular.

Mature sperm heads of a particular testicular follicle vary somewhat in length, as shown by me before ('10a). But this appears to be a case of continuous variation, and those of the same follicle do not constitute two or more constant size groups, nor do they exhibit polymorphism.

No evidence was found of the casting off of any substances by the sperm, such as has been described in mammals and in *Myxine*. Further, all the sperm of a follicle appear to develop, and when degeneration phenomena are found (such as marked vacuolization of the sperm heads) they usually include all the sperm of a cyst. There is no evidence that particular sperm degenerate, nor any particular proportion of them.

During their earlier stages the spermatozoa are arranged irregularly within their cysts. But after they have lengthened, as in the stage of fig. 126, all those of a cyst become grouped into bundles with the heads close together and directed towards the vas deferens, while their tails point in the opposite direction. After the heads have grown much longer, as in the stage of fig. 146 or a little earlier, all the spermatozoa of a bundle have the pointed ends of their heads imbedded in the cytoplasm of one of the larger follicular cells that form the thickened epithelial lining of the distal end of the testis (compare the text figure of my paper, '10a); but this attachment, which may denote a feeding process, becomes broken before the sperm arrive in the vas deferens.

### *B. Discussion*

In his classical memoir of 1883 the lamented Eduard Van Beneden gave the first statement of the theory of the individuality of the chromosomes, and at the same time determined that the mature germ cells contain half the normal number of chromosomes, two conclusions that are of the greatest importance. Since that time much attention has been given to the mechanism of these processes, and the interest in them never seemed more intense than at the present time.

My present paper gives results that are thoroughly confirmatory of the idea of the continuity of the chromosomes, a view that I have consistently tried to support. In *Euschistus* there is no rest period in the spermatocytes, but the boundaries of all the autosomes may be readily distinguished from the early growth period through both maturation divisions into the early part of the histogenesis. This is not only a continuity of number but also—and this is more important—one of size differences. This subject has received so much attention in preceding papers of mine (especially those of '00, '01, '06, '10a) that there is no gain in another discussion of it here.

On the matter of the mechanism of the reduction of the chromosomes there is a very voluminous literature, as every one knows to his cost who has tried to keep abreast of the discussion. Fortunately Grégoire ('10; compare also his earlier study of '05) has recently published an excellent comprehensive critical review of this whole literature through 1909, so that I do not need to go over the ground in this place but will refer the reader to Grégoire's work as the most thorough discussion of the question.<sup>6</sup> Below I give a concise tabulation of the chief opinions relating to the phenomena of reduction of the autosomes, arranging these in somewhat different form from that of Grégoire, and mentioning for each view simply its main founders. In this tabulation I do not include the "interprétations spéciales de nature complexe" which Grégoire reviews on pp. 269–273 of his last study; these are explanations offered by Häcker (and his students), by Schaefer, Otte, Wilke and Marcus, all of them differing rather markedly from other modern opinions, and none of them as yet particularly corroborated.<sup>7</sup>

<sup>6</sup> Grégoire seems to have omitted only one important paper on the subject, that of Guyer, which was dated 1900 but does not appear to have been published until two or three years after that time. Stomps has issued, since the appearance of Grégoire's work, a study of considerable interest upon the maturation phenomena of *Spinacia*.

<sup>7</sup> The most important of these explanations is perhaps that of Häcker ('04, '10). But his idea of a teleutosynopsis, a conjugation of autosomes during or after the second maturation mitosis, are in complete disaccord with the work of Rückert, Lerat, Nichols, McClendon and others on crustacean gametogenesis. This dis-

I. The actual reduction of the number of the chromosomes is effected during the prophases of the maturation mitoses, and both of these divisions are equational. This was originated by Boveri ('87) and Brauer ('93). To-day it is held in two forms: (A) Meves ('96, '07a) Fick ('07, '08), and Duesberg ('08) argue that a continuous spirem is produced, that this segments into half the normal number of chromosomes, the cleft along such a bivalent chromosome being a true longitudinal split; they reason there is neither metasyndetic nor parasyndetic conjugation of chromosomes. (B) Bonnevie ('06, '08) and Vejdovský ('07) hold there is a parasyndetic union of chromosomes, but that this conjugation leads to complete and persisting fusion.

II. The reduction of the number of the chromosomes is effected by the maturation mitoses, one at least of which is a reduction division. There are several variants of this, as follows:

A. The univalent chromosomes, without conjugation or pseudoreduction, double their number during the prophases then become quartered in number by two successive reduction divisions. This view was founded especially by O. Hertwig ('90) and Wilcox ('95).

B. The univalent chromosomes undergo neither conjugation nor pseudoreduction during the prophases, but conjugate first in the equator of the first maturation spindle, and there separate reductionally. Founded by Henking ('91) and Korschelt ('95).

C. The univalent chromosomes undergo pseudoreduction in the prophases by a continuous chromatin spirem segmenting into half the normal number of segments; these divide equationally in the first mitosis, and reductionally in the second; Rückert ('93, '94), Häcker ('95), Vom Rath ('95).

agreement need mean nothing in itself, but the more serious objection is that Häcker has tried to analyze the phenomena to large extent from a study of the maturation mitoses, without special regard to that most important series of stages found only in the growth period. Accordingly, Häcker's conclusions cannot be considered in any measure founded until he has filled in this gap, for every one must recognize how elusive it is to argue phenomena of change simply from the phenomena of behavior of the definitive gemini.

D. There is no continuous chromatin spirem produced in the prophases, but the univalent chromosomes conjugate to form pairs or gemini and these undergo a reductional and an equational division. This view, now shared by the great majority of students, exhibits itself under these aspects:

D. 1. The chromosomes conjugate metasyngetically.

(a.) The reduction in number of the chromosomes is effected by the first maturation mitosis (Montgomery, '00; Farmer and Moore, '03).

(b.) The reduction in number of the chromosomes is effected by the second maturation mitosis, McClung, '00, and his students.

D. 2. The chromosomes conjugate parasyngetically, and the first maturation mitosis is reductional; Winiwarter ('00), Grégoire ('04), Berghs ('04), A. and K. E. Schreiner ('04, '05).

We may now discuss the relative validity of these different views, referring the reader for a full bibliographical treatment to the review by Grégoire.

Interpretation I is founded to great extent upon negative results, and rather ignores the numerous cases where particular forms and sizes of chromosomes can be followed through different generations with a high degree of certainty. It also denies the persisting continuity of chromosomes, an attitude based entirely upon negative evidence; it argues that at certain times one cannot see the boundaries of chromosomes, therefore they must have lost their individuality. This view further gives no satisfactory explanation of why the chromosomes of the first maturation mitosis are so different in form from other chromosomes.

Interpretation II meets the great consensus of modern opinion, and I believe it to be fully established. On the four principal variants of this view the following criticisms may be passed:

The view IIA meets no modern confirmation of any description, it being well established that not more than one maturation division is reductional.

The view IIB was based on Henking's observations on *Pyrrocoris*, but all later students of hemipteran spermatogenesis have shown that gemini are produced before the maturation mitoses;

and based also upon Korschelt's account of *Ophryotrocha*, but this has been controverted by the accounts of Grégoire and Deton ('06) and of the Schreiners ('06b). Therefore this view has no longer any support.

View IIC is a much closer approximation to the truth, because it grants that gemini are produced during the growth period. The objection to it is that there is probably no continuous chromatin spirem produced in the prophases of the first maturation division nor in any portion of the growth period; this objection was first urged by me ('00) as one of the many differences between spermatogonia (or oogonia) and spermatocytes (or oocytes), receives strong confirmation in my present observations, and has been sustained by Grégoire and numerous other investigators; only Meves and his school still oppose our contention. It is not a minor but a major difference, for the segmentation of a chromatin spirem is one thing, the conjugation of univalent chromosomes into gemini quite another matter. The conclusion that like or homologous chromosomes pair together to make gemini is the only conclusion that suffices to explain why, in the spermatid or the ootid, all the chromosomes may be unlike in form or size.

Therefore we must subscribe to the view IID, that there is no continuous chromatin spirem produced, but that the gemini are engendered by the pairwise conjugation of univalents and that they undergo one reduction division. This idea of a true conjugation of chromosomes was brought out by von Winiwarter and myself independently in 1900, and in the following year I showed that this is a conjugation of correspondent maternal and paternal chromosomes, therefore really the final step in the fertilization process of the germ cells. This view IID has been substantiated, if the greater consensus of modern opinion may be taken as a test of truth.

Twelve years ago, when I wrote my first paper on *Euschistus* (*Pentatoma*), those who held to the occurrence of a reduction division, and they were but few—Rückert, Vom Rath, Häcker, Wilcox, and in opposition to the authoritative opinions of

Flemming, Brauer and Boveri, maintained the occurrence of post-reduction, *i.e.*, reduction accomplished by the second maturation mitosis. It was Henking who first gave strong evidence that the first mitosis is reductional. Since 1898 I have tried to demonstrate for a number of objects (Hemiptera, Peripatus, Plethodon, Lycosa, Syrbula, Ascaris) it is the first mitosis that effects the actual reduction in the number of chromosomes, and not the second. This too is now fairly generally accepted, and Grégoire has taken the stand that it is actually proven for the greater number of the metaphyta and metazoa that have been studied. There are not many who longer hold that the second mitosis is reductional; the chief among them are McClung and his students, but others who have investigated the spermatogenesis of Orthoptera (Gérard, Davis, Montgomery) have brought evidence to show that here too the first mitosis is reductional. Blackman agrees with the school of McClung, but then the chromosomes of the myriapods are admittedly unfavorable for the decision of this question. And it is not unimportant which one of the maturation mitoses is reductional, for in some cases, as in certain parthenogenetic eggs, there may be only one such mitosis, and it is a matter of great theoretical interest whether this is reductional or equational.

But while it has been my good fortune to find so many issues for which I have contended now so generally maintained, there is one important question in which I would appear to have been mainly in the wrong, and it is my present observations that have convinced me of this. That is the question of the mode of conjugation of the chromosomes. Here there are two main opinions. The one, founded by von Winiwarter ('00), is that of parallel conjugation of the chromosomes, known as parasyn-desis (Häcker) or parasynapsis (Wilson). This view, which now enjoys much the greater support, goes to show that after the last spermatogonial mitosis the chromosomes become very delicate slender threads, the leptotene condition (von Winiwarter; leptomena, Grégoire); these then approximate themselves parallel into pairs making the zygotene condition (Grégoire; synaptène,

von Winiwarter; amphitène, Janssens); the univalents then approximate so closely as to form thick double threads, the pachytene condition (von Winiwarter; pachynema or spirem, Grégoire); then each such thick thread becomes distinctly double again by a process of unrolling of the constituent univalents (diplotène, von Winiwarter; strepsitene, Dixon; strepsinema, Grégoire). At any period of these stages a contraction of the chromosomes may take place (synapsis, Moore; synizesis, McClung). Thus just before and after the pachytene condition each geminus may show a split along its length, which is the path of approach and retreat of the univalents and not a longitudinal split of univalents; a true longitudinal splitting of univalents does not occur until late in the diakinesis or may not appear until the anaphase of the reduction division.

The other chief view of the conjugation of the chromosomes was introduced by me, also in 1900, and is that termed by me end-to-end conjugation and by Häcker metasynopsis (telosynapsis, Wilson). I interpreted the phenomena in this sense first for *Peripatus*, later also for *Euschistus*, *Plethodon*, *Syrbula* and *Lycosa*. It maintains that shortly after the last spermatogonial mitosis the chromosomes conjugate end to end, or, in the case of ring-shaped ones, by both ends, and that subsequently (my postsynapsis stage, equivalent to the diplotene) each univalent element becomes longitudinally split. This view gives no satisfactory explanation of the pachytene condition.

The observations of the present paper are in accord with the view IID, which implies parasyndesis with prereduction. My original conclusion was right that in the species examined by me the chromosomes are paired end to end in the first maturation spindle and in the late prophase, whereas in most other cases they lie in these stages in parallel juxtaposition. But I made my error, as Grégoire has pointed out, in overlooking or giving scant attention to most of the stages preceding the pachytene condition, which are necessarily the decisive ones; I also employed too low powers of magnification for such delicate determinations. During the past year I have also convinced myself of the occurrence of

parasyn-desis in *Plethodon* such as Janssens had described for this object and the Schreiners for *Salamandra*.

The main difference between the views of parasyn-desis and metasyndesis lies in the interpretation of the longitudinal cleft of the gemini.

The question has been much discussed as to exactly what takes place during the conjugation (zygotenia) of the chromosomes; whether there is actual fusion of them, or substance interchange or simply a close interlacing. The phenomena in *Euschistus* do not indicate any fusion of the conjugants, for it is generally possible to distinguish the two components of each geminus even in the pachytene conditions. I have compared ('01) this process with the conjugation of Protozoa, and it may indeed be the same as in the conjugation of two *Paramaecia*, the two individuals being for a while so closely apposed that no line of demarcation can be seen between them, while afterwards they separate into the same two individuals as before. In the Ciliata there is interchange of substance between the conjugants, so that one would anticipate a similar process in the case of conjugating chromosomes, but this cannot be determined without instituting careful microchemical studies upon the chromosomes before and after the pachytene stage.

A minor point remaining to be mentioned is that in *Euschistus* the nucleus of the mature sperm is a cylinder of chromatin around a central core of karyolymph, and is not a solid mass of chromatin. A similar condition was noted by Baumgartner in *Gryllus* ('02). For the most part describers of insect spermatogenesis have delineated the chromatin of the nucleus as disintegrating into fine granules which later condense into a solid mass. This matter is worthy of some consideration by those interested in microchemical studies of sperm heads; for these are by no means pure chromatin, as sometimes supposed, but contain some cytoplasm, the substance of the perforatorium, and a considerable amount of karyolymph.

## THE ALLOSOMES

*A. The idiochromosomes and the discharge from the sperm nucleus*

These are those modified chromosomes called 'chromatin nucleoli' by me in 1898 and 1901, and 'diplosomes' in 1906 and 1910. They correspond exactly to the bodies termed by Wilson 'idiochromosomes,' and I believe his term is preferable to either of mine in the present instance.<sup>8</sup>

The behavior of these bodies was correctly described by me for the later growth period in 1898, their behavior during the maturation divisions first correctly elucidated by Wilson, and their main features have been later redescribed by me ('06, '10). In the present paper are presented some facts on their behavior in the spermatocytes, and more especially their relations during the histogenesis of the sperm that has not been heretofore examined.

In resting stages of the spermatogonia (figs. 1, 3,) they cannot be recognized, and there presumably compose a part of the nuclear reticulum; nuclear bodies that I had previously ('01) supposed to represent them in these cells I now believe to be the minute chromosomes (*m*).

The whole history of the spermatocytes, and particularly of the second maturation division, proves there is a pair of idiochromosomes of unequal volume, and these, together with the twelve autosomes, compose the fourteen elements of the spermatogonial mitoses (fig. 2); the smallest of the bodies is the smaller idiochromosome (*d*), but which is the larger cannot be told at this time, though it is probably one of the three next in size. Both idiochromosomes must divide in these mitoses, for all spermatocytes receive fourteen chromosomes, as we have seen while

<sup>8</sup> In '06 the word 'diplosome' was applied by me to allosomes that occur in pairs in the paternal germ cells, diplosomes being a collective term for the two sets of structures that Wilson had distinguished as idiochromosomes and microchromosomes; a monosome would be an unpaired allosome. I was well aware at that time that the name 'diplosome' had been previously employed for pairs of centrioles, but in that particular sense had fallen into abeyance in recent years.

considering the autosomes. The smaller idiochromosome is marked by the letter *d* in the early spermatocytes of figs. 5 to 9, here distinguishable by its size alone. In the cyst from which fig. 9 was made several nuclei exhibited this element in the form of a small rod, as there shown. As the autosomes become less compact and with Hermann's triple stain change from red to violet in their color, the idiochromosomes become distinguishable by remaining more dense and continuing safranophilous. Thus in figs. 13, 15, 18-20 an idiochromosome is readily discernible, but whether this single one is the large idiochromosome, the smaller in that case being hidden, or whether this is the smaller, could not be told with certainty. But from the stage of fig. 22 on, both of them can be recognized whenever the whole nucleus lies within the section. From these stages until the late prophase following they continue in contact with the nuclear membrane, but maintain no particular location upon its surface. They are at first separated from each other (figs. 22, 23, 27), but later come together (figs. 26, 28-30, 33, 34, 36-40, 42, 44, 48-56). But there is either variation in the time of their conjugation, or else it may be that after an initial conjugation they may undergo a temporary separation; thus they are seen separated in figs. 41, 46, 47. At first they are rather irregular in form (figs. 22, 23, 25, 26), then become more or less straightened (figs. 28-30, 36, 38, 39, 42); perhaps they are not so variable in form as the optical appearances suggest, for the supposed variation may be due in part to the angle of vision. Occasionally one or both may seem bilobed, perhaps an indication of a process of longitudinal splitting (figs. 42, 44-46, 48, 50, 51, 55; see also fig. 6 of my paper of '01). Each rounds up later and develops a clear vacuole within its interior (fig. 58). At the same time the two that had been rather loosely apposed become compacted together, so that from the stage of fig. 57 on until the late prophase a bivalent idiochromosome (*Dd*) is found in most nuclei (figs. 59-68, 69, 70). The bivalent idiochromosome sometimes exhibits two vacuoles (fig. 59) which evidently correspond to the two of the separated idiochromosomes of fig. 58, but more usually contains a single vacuole; this larger vacuole is then probably a fusion of the two single ones accompanying the

close juxtaposition of the two idiochromosomes. The growth in dimensions of this bivalent idiochromosome seems to be due to the amount of the vacuolar substance, for the safraninophilous periphery continues dense and compact. In the following prophases this separates again into its two components which have the same relative sizes as before their conjugation, indicating that the two had not fused. Fig. 68 shows the beginning of this separation and fig. 69 is possibly an earlier stage of it; in figs. 71-73 the two are completely separated, and from this time on the larger one (*D*) continues as before to lie against the nuclear membrane, but the smaller (*d*) comes to float within the nuclear cavity. A little previous to their separation the contained vacuole decreases in size (compare fig. 63 with figs. 64-70, 72, 73), and then completely disappears (figs. 71, 75, 78). The smaller idiochromosome never shows a vacuole after it has separated from the larger, and it appears regularly rounded, while the larger is lengthened. The smaller one (*d*) becomes distinctly recognizable among the other chromosomes by its smaller volume (figs. 83-86), and becomes constricted—probably a longitudinal splitting (fig. 85). The larger one (*D*) may be distinguished by its dense structure up to the time of fig. 81, but when the autosomes have become compact it can no longer be distinguished from them (figs. 83-86).

Their behavior during the maturation mitoses need be only briefly outlined, and for the sake of completeness, for this was fully described by Wilson. Only the smaller idiochromosome (*d*) can be recognized during the first mitosis, and by its characteristic small size (figs. 88-94, 96). It divides by itself, therefore equationally, and is usually placed peripherally in the chromosome plate (figs. 90, 91). The larger one also divides and separately from the smaller, because each second spermatocyte receives a total of eight larger chromosomes, namely, six autosomes and two idiochromosomes. Both may be recognized with certainty in the second spermatocytes, for there both are unipartite while each autosome is a dyad. Fig. 96, an anaphase of the first mitosis, shows only the smaller (*d*), but both (*D*, *d*) in figs. 97 and 98 (the latter a polar view of one chromosomal plate). In the equator of each second spermatocyte they conjugate again

(*D,d*, fig. 101, polar view), there composing a bivalent body that divides reductionally in that the larger idiochromosome goes into one spermatid and the smaller into the other (*D,d*, figs. 102, 103). Polar views of the anaphase of this second maturation division show, accordingly, the smaller idiochromosome in one spermatid and the larger in the other (figs. 105, 106).

In each spermatid the idiochromosome comes to lie in the nuclear vacuole apart from the autosomes, and differs from them by its more rounded form (*D,d*, fig. 110). The idiochromosome of each spermatid nucleus continues central (figs. 111–116), while the autosomes assume a peripheral position.<sup>9</sup> The idiochromosomes become connected with fine threads and chains of minute globules. Later they become much more irregular in form (*D*, figs. 117–127); each generally seems like an irregular rod, but often as two or three separate portions that may be sections of such a rod. In one case an idiochromosome had the appearance of a ring surrounding a granule (fig. 124). During this period also they lose their affinity for nuclear stains and appear pale. The nuclear contents appear highly variable, but the idiochromosome may always be distinguished from the threads and minute globules as well as from the peripheral chromatin.

Then follows the sudden disappearance of the idiochromosome. It first approaches the centriolar pole of the nucleus, as seen in figs. 126, 128 to 130. Then the autosome envelope of the nucleus opens widely at that pole and the nucleus undergoes a marked shrinkage in size (figs. 130 to 135). It is rarely that one finds the condition of figs. 131 and 132 with nuclear material evident in its outward passage, from which I judge the process must take place very rapidly. It is also rare to find any trace of exuded nuclear material within the cytoplasm (figs. 131–134) though I have sought for it with a variety of staining methods. It is to be noted, however, that immediately following this stage

<sup>9</sup> From the stage of fig. 115 on I have marked the idiochromosome by the capital letter *D*, though in these later stages it seems impossible to say for a particular nucleus whether it is the larger or the smaller.

half of the sphere (*Pf*) which had previously stained faintly now stains deeply, which might imply that the discharged nuclear substance had combined chemically with that portion of the sphere. Yet against such an interpretation it is to be remarked that the nuclear discharge seems always to be effected on that side of the centriole (*c*) directed away from the sphere (*Sp*, figs. 131 to 134). This matter was also studied by me in living sperm and in them I was able to find all the appearances shown in the drawings of fixed and stained material, though in life I could neither see the nucleus becoming visibly smaller nor see any substance passing out of it. Therefore the temporary withdrawal of the envelope of chromatin from the centriolar pole and the diminution of nuclear volume is an entirely normal process, in no way an artefact; and in life, quite as clearly as in sections, is to be seen the condition of fig. 135, with one pole of the nucleus quite clear as though substance had passed out of it, a condition in marked contrast to that of figs. 125 to 130. There is some doubt as to the nature of the discharged nuclear material, especially since no definite trace of it persists in the cytoplasm and there is no evidence that it is thrown out of the cell. But there is some indication that the idiochromosome is at this time either eliminated from the nucleus, or else changes its nature to such an extent that it can be no longer distinguished. For, omitting from consideration the appearances of discharge shown in figs. 131 to 134 it is remarkable that after the nucleus has shrunk in size (figs. 135 to 147) it never shows a trace of the large granular idiochromosomes. The fact is that the idiochromosome is clearly evident up to the time of the nuclear discharge, while after then it can no longer be seen.

After the discharge from the nucleus its contents are sparse achromatic globules and threads (figs. 135 to 143), later very fine granules (fig. 144), while in the mature sperm head (fig. 147) the nuclear sap appears nearly homogeneous. This nuclear shrinkage takes place on all sperm without exception.

*B. The minute chromosomes*

This non-committal term is given provisionally to these corpuscles.

In the rest stage of both generations of the spermatogonia the nucleus contains two minute dense bodies of different volume (*m*, figs. 1 and 3) that stain deeply with basic stains and may thereby be distinguished from the plasmosome (*Pl*) as well as from the general reticulum; with Hermann's triple stain they show bright red, the reticulum violet and the plasmosome brown. These were described by me in 1901, and then I sought to identify them with the idiochromosomes of the spermatocytes. But such identification must be considered erroneous, for both of these bodies are much smaller than the smaller idiochromosomes, as can be seen by comparing the latter (*d*, fig. 2) with the former (*m*, fig. 1).

On polar views of spermatogonial spindles I have not been able to recognize them with certainty, nor yet during the growth period of the spermatocytes, probably on account of their small dimensions. But in the late prophases of the first maturation division they reappear to the number of one or two, never more (fig. 86, *m*). I have never seen more than a single one in any first maturation spindle; sometimes it is the larger one that is seen (fig. 90, *m*), sometimes the smaller (fig. 91, *m*). These come to lie within the chromosomal plate (figs. 88, 90–93), never outside of the spindle, thus behaving like true chromosomes.<sup>10</sup> They lie in the equator of the metaphase of the first division, but pass undivided into the second spermatocytes (fig. 96). In the second maturation spindle they cannot be recognized, whence we may conclude that they either become attached to one of the other chromosomes, or else come to lie outside of the spindle, in which case they would be indistinguishable from mitochondria; the former alternative is probably correct, for that was found by me

<sup>10</sup> It is an important criterion of chromosomes that they are integral parts of the spindles, while yolk globules, chromatoid corpuscles and mitochondria always lie outside of the nuclear portion of the spindle—the portion composed of the mantle fibers that are directly derived from linin.

in other testes ('10a). I have made some counts to determine their constancy. In testis no. 265 one was seen in seventeen out of thirty polar views of the first maturation metaphase, but none in sixteen polar views of the second; in testis no. 282 one was observed in seven out of twenty-eight polar views of the first maturation, but none in twenty-seven polar views of the second.

### *C. Discussion*

There are two kinds of allosomes, the minute chromosomes and the idiochromosomes.

The minute chromosomes would appear to be allied to the very small chromosomes called by Wilson the microchromosomes of such forms as *Anasa*, but they differ from them in not dividing in the maturation mitoses. Minute chromosomes were described by me before ('10a) in another testis (no. 120) of the same species, and there they were frequently attached to other chromosomes during the maturation divisions: they were then termed by me 'supernumerary' chromosomes. On account of their minute volumes little can be ascertained of their behavior, but the fact that they do not appear to divide might indicate that they are degenerating idiochromosomes.

The idiochromosomes proper are not recognizable in the resting nuclei of the spermatogonia, for what I had previously ('01) supposed to be idiochromosomes in these cells I now find are the minute chromosomes. The idiochromosomes thus come to behave differently from the ordinary chromosomes first in the spermatocytes, in agreement with my earliest account of 1898.

One of the most interesting facts determined is the discharge of a considerable volume of material from the nucleus during the histogenesis of the sperm. A sudden shrinkage of the sperm nucleus just before it begins to elongate has been described or figured for a considerable number of insects, thus by Henking ('91), Gross ('04, '06), Paulmier ('99), Buchner ('09), Wassilieff ('07), Davis ('08), McGill ('06), Cook ('10), Jordan ('08), Pantel and de Sinéty ('06), Boring ('07), while Otte ('07) has called particular attention to it. It would then appear to be a common

phenomenon in insects, and judging from Meves' ('00) figures of *Paludina*, the sperm head would appear to shrink there also. In *Euschistus* it is at about this stage that the idiochromosome, up to that time very distinct in the centre of the nucleus, disappears from view, either it is then discharged from the nucleus, or else it then becomes so changed physically that it can no longer be recognized. Now we know that allosomes invariably enter into the composition of the sperm nucleus, and generally occupy a position near its middle, from the studies of Henking, Gross, Otte, Buchner, Gérard ('09), Davis, Paulmier, Stevens ('05, '06, '10), Boring and Wallace ('09). Some of these investigators have been able to trace the allosome only during the earlier part of the histogenesis of the sperm, and so far as I can learn no one has been able to identify it within the mature sperm. But some have been able to distinguish it within the sperm head after the nucleus has contracted, as Henking, Gross ('06), Jordan, and Otte, and we know positively from the studies of Morrill ('10) that the sperm carries allosomes into the egg. By analogy with these cases it may be that the idiochromosome of *Euschistus* remains within the sperm nucleus even after the latter has discharged substance, but whether it does or not it certainly disappears from view at that time.

## THE PLASMOSOMES

### *A. Observations*

These were correctly described by me in 1898 and there is little to add to that account.

In the spermatogonia one or two occur in the resting nucleus (figs. 1 and 3). In the spermatocytes they may be readily distinguished from the idiochromosomes by their staining reactions and form: they stain brown or pale violet and the idiochromosomes red after Hermann's triplestain, and, after Delafield's haematoxylin and eosin, they stain red and the idiochromosomes blue. With iron haematoxylin they may either stain as deeply as the idiochromosomes or paler, dependent upon the degree of extraction

of the stain. Both may be readily distinguished in life during the growth period.

In the spermatocytes they are almost invariably single, rarely double, in number, and appear first as flattened discs upon the inner surface of the nuclear membrane (figs. 8, 14). Generally they are not recognizable at such early stages, usually not until about the commencement of the leptotene condition. They maintain this peripheral position, increasing in size until well into the strepsinema stage (figs. 26, 29-33, 35, 37-39, 42, 43, 45-47, 50-52).<sup>11</sup> It is to be noticed that their exact position upon the nuclear membrane is quite variable, save that they are rarely near the chromatin plate and never in contact with the idiochromosomes. They later change this position to become free within the nuclear cavity (figs. 53 to 68). In the prophases of the first maturation division they become smaller and gradually disappear without a trace before the autosomes have taken their definitive forms (figs. 69 to 73 and 75). No plasmosomes seem to be produced during the histogenesis of the sperm.

During the growth period there are certain constant relations of the plasmosomes to the autosomes. As long as the plasmosomes remain against the nuclear membrane they are in contact with radiating strands of the chromatin (autosomes), as shown in figs. 14, 22, 26, 29-33, 37-39, and most frequently the condition is that of the chromatin ending with a broadened plate upon the surface of the plasmosome (figs. 22, 29, 32, 37, 39); thus the plasmosome frequently appears much darker than it really is by reason of the covering of chromatin. Usually not more than two autosomes make this connection, frequently only one, and this connection is the more noticeable because, during these stages, autosomes have no other regular regular peripheral attachment except with the chromatin plate that lies next the idiozome. When the plasmosome has grown much larger it loses its connection with autosomes (figs. 42 to 52); and after this contact has become broken the plasmosome continues to increase in volume, so that its growth

<sup>11</sup> In the nuclei of figs. 47 and 50 they appear to lie free within the nuclear cavity, but this is simply because they are not seen in profile.

cannot be dependent upon such contact. After it has moved into the nuclear cavity it establishes a second connection, this time with a number of autosomes (figs. 58, 60 to 63), a connection that becomes lost when the autosomes take their places upon the nuclear membrane and leave the plasmosome behind them. It is just at this time that the plasmosome reaches its maximum size and shows the most irregular form. As the autosomes separate from it, which commences at the stage of fig. 63, the plasmosome becomes much paler and remains pale until it finally disappears by dissolution.

Thus the plasmosome of the spermatocytes has a twofold connection with the autosomes, first during its early growth, and second during the time of its greatest volume.

#### *B. Discussion: Genesis and kinds of nucleoli*

Though Foot and Strobell ('09) have denied the concomitant occurrence of idiochromosomes and plasmosomes in spermatocytes of this species, I believe my observations of 1898, confirmed by the present ones, settle the matter beyond a doubt.

The plasmosomes of all cell generations examined disintegrate during the prophases of mitosis, and thus are not persisting cell organs. In the spermatocytes they arise in close contact with the nuclear membrane, and at the same time in connection with the end of an autosome, which would suggest that the plasmosome is either the joint product of chromatin and cytoplasm, or else represents substance taken up by the nucleus from the cytoplasm—this latter view being expressed by me in 1899 after a study of the genesis of plasmosomes in young oocytes of *Nemertini*. These phenomena would indicate that the first produced portion of the ground substance of the plasmosome may be derived from the cytoplasm, and that this process may be directed by a particular autosome, for the plasmosome is not produced at any point where chromatin touches the nuclear membrane, thus never in the region of the chromatin plate that lies at the idiozome pole. However, it is to be noted that in *Euschistus* the plasmosome undergoes its greatest growth after it had

sunk into the nuclear cavity. Chubb ('06) has criticized my view that the ground substance of the plasmosome may be of cytoplasmic origin; but then he was admittedly unable to trace its first origin in oocytes of *Antedon*, and considered it especially in later stages when it has a chromatin envelope.

After the plasmosome has separated from the nuclear membrane it becomes for a second time connected with the autosomes, and in such a way that the latter radiate towards and end upon its surface; in its later history, accordingly, it would seem to stand in closer metabolic relation with the chromatin than before. Maziarski ('10), in a detailed study of the plasmosomes of gland cells, considers them to have the function of elaborating true chromatin to take the place of that eliminated into the cytoplasm.

It may be of service to characterize and define the various kinds of nucleolar structures of metazoan cells, for there is still much confusion regarding them. As 'nucleolar structures' are here meant all the larger, generally more or less spherical, inclusions of the vegetative nucleus, with the exception of the two following that are of strictly chromosomal nature: karyosomes, which are thickenings of the nuclear reticulum, and allosomes, or modified chromosomes. Leaving out of consideration the karyosomes and allosomes, nucleolar structures may be classified as *nucleoli* and as *karyospheres*.

Nucleoli are bodies that do not contain the nuclear reticulum, and from which chromosomes do not emerge. They may be subdivided into three groups, according to the distinctions made by Carnoy in 1884 which the modern researches are tending to corroborate. (1) The 'plasmosomes' of Ogata, correspondent with Carnoy's 'nucléoles plasmatiques' and my true nucleoli; these are always acidophilic. (2) 'Chromatic nucleoli' or 'chromatoids,' correspondent with Carnoy's 'nucléoles nucléiniens;' they take chromatin stains, sometimes more intensely than the chromatin, but often with a different tone of color. It is only by a study of their history and fate that we can distinguish between these and karyosomes. Chromatoids are often opposed to plasmosomes to compose so-called 'double nucleoli,' as in germinal vesicles of certain annelids, molluscs and arthropods.

(3) Mixed nucleoli, those composed of a mixture of plasmosome and chromatoid substance; Maziarski and others consider these an intermediate stage between the other kinds of nucleoli.

We will adopt Blackman's term of 'karyosphere' to denote compounds of nucleoli, of whatever kind, with chromatin reticulum or chromosomes. Karyospheres are therefore containers of some or all of the chromosome substances, and correspond in part with Carnoy's 'nucléoles-noyaux.' In the germ cells they are limited mainly to primary oocytes and spermatocytes. The following kinds of them are known:

(1) Combinations of plasmosomes and allosomes, in which the latter are more generally apposed to, more rarely imbedded in, the plasmosome. These are frequent in insect spermatocytes, especially in Hemiptera. They are separate from the nuclear reticulum, and from autosomes.

(2) Associations of plasmosomes with both autosomes and allosomes, best known in the spermatocytes of myriapods after the researches of Blackman ('03, '05, '07) and Medes ('05). These may at certain stages contain all the staining substance of the nucleus. In some myriapods the plasmosome constituent would seem to be lacking. They are the most complex of all nuclear structures.

(3) Associations of autosomes or ordinary chromosomes with a plasmosome, which differ from the preceding kind in lacking allosomes. These are known in germinal vesicles of certain echinoderms, mammals, araneads, and *Paragordius*, and spermatocytes of certain insects. Their genesis is as follows: A nucleolus develops, more or less of the nuclear reticulum moves into it, and in the prophases of mitosis chromosomes emerge from the nucleolus.

As incipient karyospheres of the third kind might be considered cases where chromosomes terminate against nucleoli without being wholly enclosed in them. Such are the chromoplasts of *Batrachoseps*, according to Eisen ('00) and Janssens ('05); and such also would be the nucleolar relations we have described in *Euschistus*.<sup>12</sup>

<sup>12</sup> Nichols ('10) has tried to relate the different kinds of nucleolar complexes, and refers them all to the common physiological basis of a transfer of chromatin material to, and a later withdrawal of it from, the plasmosome.

## THE CHROMATOID CORPUSCLES

These are the bodies that were called yolk globules in my paper of 1898. They are rounded bodies within the cytoplasm, which stain in general like the plasmosomes, *i.e.*, red after Delafield's haematoxylin and eosin, and red after the Ehrlich-Biondi triple stain, with which the chromatin stains blue and green respectively. But with the triple stain of Hermann they color with safranin, while the plasmosomes are pale violet, and with iron haematoxylin the latter stain intensely black. At the time of their first appearance in the first spermatocytes they lie usually near the nuclear membrane (*ch. c*, figs. 59, 63). Through the prophases of the first maturation mitosis they are regularly seen (figs. 65, 66, 71, 85). During the maturation mitoses they lie outside of the spindle and do not divide (figs. 87-89, 92, 93, 95, 99-104, 107, 109, 111). In the second mitosis it is not uncommon to find several of them in one daughter cell and none in the other (figs. 104, 107-109), or again they may be found in both, thus their apportionment to the spermatids seems to be a matter of chance. During the histogenesis of the spermatids they occur in variable positions (figs. 111, 112, 115, 116, 117-120.) Later than the stage of fig. 124, which shows one close against the nucleus, I have not been able to see them.

The name 'chromatoider Nebenkörper' was introduced by Benda ('91) for similar bodies in mammalian spermatocytes. They have been more fully treated especially by Lenhossék ('98), the Schreiners ('05, '08) and Meves ('99). The Schreiners have reviewed the literature, and find that in *Myxine* the chromatoid corpuscle is produced by the fusion of several 'chromatoid nucleoli' (distinguishable from paler nucleoli) that emigrate from the nucleus of the first spermatocyte; this corpuscle persists in the cytoplasm into the spermatid, and in the latter eventually wanders back into the nucleus. They were the first to have described its nuclear origin and nuclear return, while Meves had previously ('02) suggested they might be discharged plasmosomes.

## CELL AXES, CENTRIOLES, FLAGELLA

Each spermatogonium is distinctly bipolar (figs. 1, 3), with one pole occupied by the nucleus and the other by the mitosome and the greatest mass of cytoplasm; the long axis of the cell passes through these two.

During the earlier growth period the spermatocytes show corresponding poles, which may be named, in accord with my previous terminology ('00), the central and the distal poles; the nucleus lies nearest the former, and there was also the centriole in the early anaphase of the last spermatogonial division, while the greater mass of cytoplasm and the idiozome lie at the distal pole. This constant polarity persists from the stage of fig. 5 to that of fig. 58. The primary axis of the first spermatocytes is then a line joining these two poles, and the axis of the first maturation spindle comes to lie at right angles to it, while that of the second maturation spindle coincides with it.

The centrioles of the spermatogonia (fig. 4) are exceedingly minute, and I have not restudied them with any particular attention. They were rather fully described in my paper of 1898, but with too low powers of magnification for exact demonstration. They could not be seen in the spermatocytes until the idiozome had disintegrated. During the earlier growth period it is probable they lie within the idiozome, judging by analogy with the phenomena in other animals and for reason of the following facts. In figs. 46 and 47, illustrating the separation of the halves of the disappearing idiozome (*id*), the nucleus is pointed at the idiozome pole; and in fig. 50, which shows the remains of the idiozome far separated from each other, the nucleus exhibits two correspondent projections. Such nuclear projections in these cells indicate the presence of centrioles, even though these are invisible, for in later stages, when centrioles become visible, the nuclear projections are always directed towards them. Therefore it follows from the appearances just described, that the centrioles have moved from the central pole of the nucleus to occupy a place within the idiozome at the distal pole, and that the centrioles leave this position within the idiozome in the primary

axis to take places to the right and left of this axis, carrying the remains of the idiozome with them. The earliest stage showing the centrioles is given in fig. 54, showing the centriole (*c*) upon the cell membrane; this cell contains still a trace of the sphere (*Sp*) but no remnant of the idiozome. Subsequently a pair of centrioles is found to the right and another pair to the left of the primary long axis (*c*, figs. 57–59).<sup>13</sup> A line joining these centriole poles is at right angles to the previous primary axis, as seen by comparing figs. 57 and 58 with fig. 52. Each pair of centrioles lies in contact with the cell membrane, at the base of an indentation; a line joining the two centrioles of a pair is usually oblique to the surface of the cell at that point. No flagella were found attached to the centrioles of the spermatocytes, not even in cells examined in life.

As the pairs of centrioles come to occupy the positions described, two conspicuous movements of cell parts result, as one finds on examination of figs. 57–59, 61–63, 65, 66, 71, 84, 85. The first of these is that the original distinction between central and distal poles becomes more or less obliterated in that the cytoplasm mass becomes almost as great at the former as at the latter pole, or in other words, the nucleus becomes more central. The second is that the nucleus becomes elongated in the direction of a line joining the centriole poles. Thus the nucleus assumes a marked ovoid outline, as seen best in figs. 59, 61, 63, 66, 71, 84, 85, with its ends pointed towards the centriole pairs. Therefore the centrioles exert a powerful attractive influence upon the nucleus, before any spindle fibers can be seen.

The centriole pairs maintain their positions on the cell membrane until the stage of fig. 84, after which they sink into the cytoplasm.<sup>14</sup> Fig. 85 shows one centriole pair still upon the surface, the other within a cytoplasmic knob, and fig. 86 shows both pairs close to the nucleus. Fig. 86 is interesting in showing a persisting primary axis, with the centrioles to the right and left

<sup>13</sup> In my previous studies centrioles were not found in these cells until the late prophases.

<sup>14</sup> In fig. 65, I could not be certain whether the centrioles lay upon the surface or beneath it.

of it, the central pole being uppermost and the distal lowermost in the drawing. When the first maturation spindle is established each centriole pair occupies one pole of the spindle and lies deep within the cytoplasm (figs. 87-89, 93-97). At the close of this mitosis the two centrioles of each pair, without dividing, move apart from each other, as described in detail in my paper of 1898, to constitute the poles of the second maturation spindle (figs. 102, 103). In this second mitosis, accordingly, each spindle pole has but a single centriole.

During the anaphases of the second maturation division (figs. 104, 106-111) the centrioles become exceedingly small so that it is difficult to see them. But in each early spermatid a minute centriole may be seen at the central pole of the cell (*c*, figs. 112, 113) in contact with both cell and nuclear membrane. To each centriole is attached a short flagellum that projects from the cell surface. The centriole then moves with its flagellum around the nucleus to the distal end of the latter, figs. 114 to 120, exhibiting successive stages of this movement. As the centriole passes along the nuclear surface it rapidly increases in size, and its flagellum grows in thickness and length. Thus the centriole comes to lie against that part of the nuclear membrane which lacks the chromatin envelope, at the base of a small pit formed by an invagination of the cell wall. Such spermatids are distinctly bilateral, with the central pole occupied by the sphere (*Sp*), the distal by the mitochondrial body, and the centriole in a plane separating a right and left side; figs. 118 and 110 show spermatids from lateral view, and fig. 119 one from dorsal or ventral aspect. As the mitochondrial mass elongates and becomes divided lengthwise (figs. 121-123) the flagellum come to extend from the centriole backwards between the mitochondrial moieties to project free at the distal end of the cell. I could not determine whether the flagellum thereby sinks into the cytoplasm, or whether it becomes placed within a longitudinal groove which closes later: but the latter is more probable, judging from the conditions shown in figs. 118 and 120, which indicate a groove along that side of the cell nearest the mitochondrial mass. So soon as the flagellum comes to lie between the halves of the mitochondrial

body, it occupies the long axis of the spermatid, and from now on may be termed the axial thread. This thread is now a fibril which in life exhibits no vibrations but is extended out straight from the cell, in marked distinction to the motile pseudopodium that will be described later; it has been drawn bent in figs. 121–126 simply to save space in the drawings, but in life such bending is not seen. It would appear at this time to be of the nature of a skeletal rod to give firmness to the growing tail of the sperm. I have not drawn the whole tail of the sperm later than the stage of fig. 126 because it soon reaches a great length. But the axial thread can be recognized from this time up to the mature sperm (fig. 147) as a fine, deeply staining line extending from the head of the sperm backwards along the tail, enclosed in a mantle of mitochondrial substance.

In the stages of figs. 127–137 the centriole (*c*) is somewhat excentric; the axial thread is attached to one edge of it, on its opposite side lies the sphere (*Sp*). In figs. 121 to 125 the sperm is shown in dorso-ventral aspect, in lateral aspect in the other drawings.

The centriole grows rapidly and becomes transversely elongated (figs. 124–137), and stains deeply throughout this period. Then it assumes a more irregular form (figs. 129, 131, 134, 136) as though it had divided into a plate of two or more smaller portions, but I have not been able to assure myself of the presence of distinct parts. Later it shifts the position of its long axis and penetrates somewhat into the nucleus (figs. 140, 141). Then it suddenly changes in appearance, showing one of two clear vacuoles, becoming very refractive; at the same time it appears constricted and pushes further into the nucleus (figs. 142, 143). In a stage slightly later (fig. 144) a single, rather small, hollow centriole is seen within a clear space in the nucleus. In later stages (figs. 146, 147) no centriole can be found. It is difficult to interpret this sudden seeming disappearance of the centriole. The conditions of figs. 142 and 143 would indicate that it is undergoing division, with a vacuole in each moiety. In the next stage the anterior portion would seem to have moved still further into the head. The other half may originate that lightly staining body placed in the head

at its junction with the tail (figs. 144, 146), which may sometime be recognized in the mature sperm.

Without relation to either centriole or axial thread is developed a cytoplasmic process or pseudopodium at the distal pole of the spermatid (figs. 114-116; 117-124). A similar structure does not seem to have been described for any other flagellate sperm. I was unable to see it in fixed material, but in living cells it is most apparent, and indeed both it and the axial thread were added to my drawings from the study of living material. This pseudopodium has some resemblance to that of a Heliozoan, beating slowly back and forth in the medium, bending and straightening. It is considerably thicker, especially at the base, than the axial thread. I could not find it in either living or fixed material later than the stage of fig. 124, so that it would seem to be a temporary organ of locomotion which may be of service in grouping the immature sperm into bundles.

#### CYTOPLASMIC STRUCTURES

##### *A. Mitosome and cell plate*

All the spermatogonia possess at the distal end a body that is probably a derivative of connective spindle fibers of a previous mitosis, and therefore is probably a true mitosome; I have not studied its genesis, but have judged rather by analogy with other species. This is lettered *mit.* in figs. 1 and 3. It is denser than the rest of the cytoplasm, finely granular, and the mitosomes of contiguous cells are frequently fused so that 'cell couples' result. It is most evident during the rest stage but persists into the succeeding prophases of mitosis. No mitosomes were seen in spermatocytes or spermatids.

Equatorial cell plates appear to be formed after each spermatogonial mitosis (fig. 4), as well as after the first (fig. 100) and second (figs. 111, 112) maturation divisions. These are granule plates developed at the point of final separation of two daughter cells. They are of relatively short duration.

*B. The idiozome*

The idiozome of the spermatogonia (*id*, figs. 1, 3) may lie against any region of the nucleus, and fig. 3 illustrates how variable its position may be. But there is here an interesting relation not noted in my previous studies. No matter where the idiozome is situated, it always touches the nucleus at that wall where the chromatin plate is; the nature of this plate was mentioned with the description of the autosomes. Therefore this idiozome probably stands in some intimate chemical relation with the chromatin plate. The idiozome is irregularly granular and becomes browned after osmic acid fixation but does not stain to any degree with any of the stains employed. It disappears during the prophases of division by breaking into smaller granules, that seem to be identical with the small bodies lying outside of the spindle (figs. 2, 4). In resting cells are found smaller scattered granules in addition to the large idiozome (figs. 1, 3), and these smaller granules may be either the remains of a previous idiozome or else mitochondria.

The young spermatocytes at the end of the last spermatogonial division (fig. 5) show no large idiozome, but merely scattered granules that may be the remains of the previous one. Very early, however, an idiozome develops in them, and throughout its existence it constantly maintains a position at the distal pole of the nucleus just where the chromatin plate lies. Fig. 6 shows the first appearance of the idiozome in a spermatocyte, and the succeeding drawings to fig. 36 represent its appearance up to the time of its beginning disintegration. Frequently it seems distinctly paired, and then each of its portions seems to be related to a particular one of those autosomes that make the chromatin plate; this relation is seen in figs. 8, 11, 13, 21, 25, 28, 34. Perhaps it is always paired but appears so only when seen from a particular pole. The figures also show that the spatial relations of the idiozome are rather closely related to the spatial extent of the chromatin plate, which would indicate growth of idiozome substance within the cytoplasm corresponding to the area of the nuclear wall involved in the nuclear chromatin plate. But there

is no evidence that chromatin particles leave the nucleus in the region of the idiozome.

At about the time the sphere arises in the spermatocytes the idiozome begins to disintegrate, whereby it becomes more coarsely granular and these granules become dispersed. Successive stages of this process are exhibited in figs. 35, 37, 39-41, 42-48, 50. During these changes the idiozome separates into two masses that, becoming smaller, move apart from each other to the right and left of the primary axis of the cell (figs. 39, 43-47), and finally come to lie near opposite sides of the nucleus (fig. 50). This movement, as we have seen, is probably associated with movements of centrioles, though the latter are not yet visible. In stages later than fig. 50 no remains of the idiozome are to be seen. During this time also the chromatin plate of the nucleus becomes disestablished, by the chromosome ends withdrawing from the nuclear membrane (figs. 42-47, 50). No idiozome body reappears in any later cell generation, nor is another chromatin plate established in the nucleus, which would show that the growth of the idiozome is determined by the chromatin plate.

### *C. The spheres*

This noncommittal term is here used for two sets of structures, both independent of the idiozome, the one in the first spermatocytes and the second in the spermatids.

In the first spermatocytes a sphere (*Sp*) is first seen in the stages of figs. 36 and 37 about the time when the idiozome (*id*) begins to degenerate. This is a rounded body at the distal end of the cell, which at first may appear either darker or lighter than the idiozome, and may touch or be separated from the latter. It lies within a large cytoplasmic vacuole; indeed during the whole growth period the cytoplasm is markedly vacuolar. The sphere increases in size until it attains its maximum volume shown in figs. 36 to 48, after which it diminishes, becoming gradually paler in color and more difficult to see (figs. 49-54). It disappears right after the stages of figs. 55 and 57. Rather rarely two spheres

are seen in the same cell (fig. 43), which then have a combined bulk equal to that of a single sphere of the same stage.<sup>15</sup> These spheres become slightly browned by osmic acid but are not affected by nuclear stains, consequently they are readily overlooked; they are seen clearest in material preserved in Flemming's solution for twelve hours or more. In consistency they are nearly homogeneous, but contain small darker particles (figs. 40, 43-45) during the time of their growth.

These spheres of spermatocytes have no conjunction with centrioles, nor do they touch the nucleus. They seem also to be independent of the idiozomes. Their relations to the mitochondria will be discussed later.

During the histogenesis of the sperm another sphere arises and passes through a rather complicated history. This is a vesicle, first noticed at the stage of fig. 114 (*Sp*), and is very pale in color after all stains; it is represented as too dark in all the figures. Its genesis was not determined. At first it lies to one side of or even behind the nucleus (figs. 114-117), after which it moves in front of the nucleus (figs. 118-124, 126). Then it passes behind the nucleus again (figs. 125, 127-134), perhaps carried by the flowing of the cytoplasm along the axial thread. It possesses at the start a clearer central vacuole (figs. 114-117) later this larger vacuole becomes excentric and then lies in that part of the sphere that touches the nucleus (figs. 119-120, 122-134). Within this larger vacuole may generally be found a minute body which stains like the ground substance of the sphere (figs. 118, 122, 126-134), and is perhaps comparable to the acrosome of other animals. At the opposite end of the sphere a smaller vacuole develops (figs. 119, 124, 127-134); it is frequently difficult to see these two vacuoles plainly. Thus the sphere comes to lie behind the nucleus, and in a definite position, namely,

<sup>15</sup> All these relations have been described as found in a particular testis, no. 282, in which a single sphere is the rule. But in certain other testes multiple spheres are frequent, so that sphere relations vary in different individuals of the same species. It is remarkable that in the large spermatocytes of follicles 1 and 3 no spheres were found.

to one side of the centriole, while at the opposite edge of the centriole the axial thread is rooted (figs. 127-134). By reason of the inclusion of two vacuoles of different sizes the sphere comes to show a bilobar form with an annular constriction, and just after the occurrence of the nuclear discharge it divides into two (figs. 135, 136). The posterior portion (*Sp.*) becomes still paler and wanders into the tail of the sperm (figs. 135-139), often passing a considerable distance behind the head (a greater distance than shown in any of the figures); there it becomes more and more indistinct, but I do not know whether it disappears entirely. The anterior portion of the sphere (*Pf*, fig. 135) suddenly becomes deep staining at the time of its separation from the posterior part, perhaps by addition to it of some of the material discharged from the nucleus. It separates completely from the posterior portion (fig. 136, *Pf*), then passes forward along the nuclear surface (fig. 137) until it arrives at the anterior end of the nucleus (figs. 138, 139). There it becomes gradually elongated (figs. 140-144, 146) and thus comes to compose the lance or perforatorium of the mature sperm (fig. 147). The mature lance is frequently thread-like and branched, nearly pseudopodial, and penetrates into the cytoplasm of the follicular nurse cells. I could not determine whether there is a cytoplasmic envelope around it and the nucleus of the mature sperm.

This sphere of the spermatid resembles that of the spermatocyte in optical appearance and in having no connection with centrioles; but differs in its elaboration of vacuoles, in its changes of form, in its division, and especially in having a close contact relation with the nucleus. The evidence is that these two spheres are dissimilar structures.

#### *D. The mitochondria*

These bodies had been figured in my paper of 1898 and described for the growth period of the spermatocytes, under the name of the idiozome mass. But I did not distinguish them from the idiozome and sphere, nor did I possess suitably stained slides for their

more thorough analysis. In the present paper I am able to describe them in much greater detail, thanks to the use of stronger powers of magnification and especially to certain very successful iron haematoxylin preparations.

In the spermatogonia no evidence was found of indubitable mitochondria. One sees there (figs. 1, 3) scattered granules in addition to the mitosome (*mit*) and the idiozome (*id*), but these have the same optical appearance as the idiozome material and therefore are probably remains of a disintegrated idiozome of a previous generation: and on sections that show the mitochondria of the spermatocytes deeply stained, these granules of the spermatogonia always remain pale. Further, there are certainly no filamentous mitochondria (chondriokonts) in the spermatogonia; from which we should conclude either (1) that there are no mitochondria at all in the spermatogonia, or else (2) that the mitochondria of these cells differ chemically and physically from those of the spermatocytes.

In the earliest stages of the spermatocytes (fig. 5) are found merely similar pale granules. But shortly thereafter there arise delicate deeply staining threads in the vicinity of the idiozome (fig. 7). They do not undergo any marked increase up to the zygotene stage (figs. 7-30), and are often apposed to the idiozome but whether actually within it is hard to determine. Sometimes, however, deep staining granules do lie within the idiozome (fig. 30). There is no evidence in these earlier nor in later stages that they are produced by emigrated chromatin particles, in the sense that chromatin particles leave the nucleus bodily, and at first they are generally separated from the nucleus. Further, they are separated from the chromatin plate of the nucleus by the idiozome. Just before the idiozome begins to degenerate they increase in length and number (figs. 31-35). Their rapid growth coincides more or less with the development of the sphere in the cell body, and with the conjugation of the autosomes in the nucleus (figs. 36-55), and they gradually come to extend into all regions of the cell body. Some of them are then usually on and within the sphere, others in contact with the idiozome, the most of them

separated from both of these bodies. Thus the mitochondria become scattered throughout the cell body but most abundantly in a perinuclear zone, as shown in figs. 42-55, 57-59, 61-63, 65, 66, 71, 84, 85. So far as could be determined they are unbranched threads, though often twisted and angularly bent. What their number is was not ascertained, the drawings representing with fidelity only the majority of those present in a particular plane of some thickness and only those seen with the greatest distinctness. Thus they come to make a thick mesh. In general they are bent around the nucleus, and mitochondria that appear on the drawings to terminate against the nuclear membrane do not really end so but curve around its surface. When the centrioles become visible the mitochondria in their vicinity frequently converge towards them (figs. 57-59, 61-63, 65, 66, 71, 84, 85). Very often they are so closely looped upon themselves that the enclosure of such a loop may present the appearance of a granule with a sharp bordering line, and this illusion is the more striking in the cases where the mitochondria are faintly stained; such appearances misled me in 1898 into supposing the mitochondria to be a mass of large granules. But when they are deeply stained it can be determined that there are no large granules in the cytoplasm except the chromatoid corpuscles.

During the prophases of the maturation divisions they become, for the most part, smooth, continuous threads, though some may retain the earlier appearance of chains of granules. Each thread is not always of even thickness throughout but frequently is irregularly dilated, as I have shown in the drawings.

I have looked to see whether there occurs in the mitochondria any process analogous to the process of pairing of the chromosomes, but have found no evidence of it.

The stages just described were worked out mainly upon one preparation, for the reason that this was most excellent for the examination of the sphere. The stages next to be described have been drawn from the sixth follicle of testis no. 265 which exhibited more clearly than any other the mitochondria during the maturation mitoses. In this testis they also appeared uniformly of

greater diameter than in any other preparation, probably because they were less strongly destained.<sup>16</sup>

Fig. 86 represents a late prophase of the first maturation division, showing the greater number of mitochondria present, and a number of these lie close to the outer surface of the nuclear membrane (not within the nuclear cavity as the figure might indicate). In this and the next following stage (fig. 87) they show no such radial grouping around the centrioles as was to be seen in earlier stages (figs. 71 and 85). In fig. 88 the first maturation spindle is established, and now as in all later stages the mitochondria lie outside of the mantle fibers; their peripheral disposition is better seen in the polar view represented in fig. 91, where only those parts of them are drawn that lie in the equatorial plane. Figs. 89, 92-94, show other cells of the same stage and demonstrate how manifold the arrangement of the mitochondria may be and how they do not appear to be influenced in any way by the spindle or the centrioles.

It would seem possible to count them in the stages of the first maturation, for at this time they are unusually distinct and large. But this is a matter of great difficulty on account of their great length and irregular twisting; thus on several occasions I have spent several days drawing those of a single cell. Further these cells are generally too large to lie wholly within the plane of a section, therefore it is not always possible to determine whether apparent ends of mitochondria may not be truncations by the

<sup>16</sup> The sections of testis no. 265 showed the mitochondria deeply stained only in follicles 1 and 6, those on the periphery which are most affected by the fixative (Flemming's fluid) while in the intermediate follicles the mitochondria were only slightly stained, also in thicker sections they are better stained than in thinner ones. This illustrates how much a matter of accident it is to secure suitably stained preparations. Sometimes it happens that of two cells lying within the same cyst, one exhibits the mitochondria very distinctly while the other does not. Or again, certain of the mitochondria within a cell stain deeply and others faintly, or one mitochondrial thread may be distinct in one portion and pale in another, evidently dependent upon depth beneath the surface of the section. I have endeavored to select for drawing only those cells in which the majority of the mitochondria appeared deeply and uniformly stained, which has required the close comparison of a very large number of cells.

knife. The counts made on the mitochondria of these figures resulted as follows:

Fig. 87, one hemisphere of a cell, about 10. Fig. 88, less than one hemisphere of a cell, 9. Fig. 89, nearly a whole cell, about 16-17. Fig. 92, nearly a whole cell, apparently 9. Fig. 93, one hemisphere of a cell, 11-12. Fig. 94, one hemisphere of a cell, 10.

These counts give from 9 to 16 or 17 distinct and separate threads. These cells were selected as the clearest in my preparations, the ones showing the mitochondria stained most deeply and uniformly, and of them that of fig. 92 was the most distinct.

There now comes up the question how these behave during the division of the cell body, for they lie wholly outside of the spindle and do not fall under its dominion. Figs. 95 and 97 each show the mitochondria of about one hemisphere during the anaphase of the first maturation mitosis; a number of them lie more or less parallel to the spindle, others lie near the poles, but none are yet dividing. In the later stage of fig. 99 all the more strongly stained mitochondria of the cell are drawn, but there is still no division of them. In fig. 100, the only good case of this stage with mitochondria well stained, the more intensely stained ones have been drawn, and it can be distinctly seen that certain of those which lie parallel to the spindle are breaking into two at the equator, and that the others which lie removed from the equator are undergoing no division at all. It thus results that mitochondria which happen to cross the line of constriction of the cell body become there broken into two, and that the others pass bodily without division into one or the other of the second spermatocytes. This happens because they are quite unsymmetrical in arrangement and do not come under the influence of the spindle fibers. Further, it would be extremely improbable that a particular mitochondrial thread that does become divided would be pinched through in its exact middle portion. Again, the mitochondria often appear more numerous at one pole of the cell than at the other. (figs. 93, 97, 99). Therefore during the first maturation mitosis there is no mechanism except the constriction of the cell body to divide the

mitochondria, consequently there is a variable and irregular portioning of them to the daughter cells, and it is a matter of chance how much mitochondrial substance goes into each of the latter.

Fig. 101 shows them on a polar view of the equatorial plane of a second spermatocyte. Fig. 102 shows all the threads (12) of somewhat more than one hemisphere, and fig. 103 all (10) of one hemisphere of the same mitosis; in these metaphases most of the mitochondria are shorter than in the preceding division because most of them had been divided then, but they lie quite as irregularly in the cell. Succeeding anaphases are exhibited in figs. 104, 107-109, illustrating that here the phenomena are essentially of the same kind as in the preceding mitosis, namely, those mitochondria that happen to lie across the equator become broken there by the cellular constriction while the others are not divided. In figs. 104 and 109 the mitochondria of only one hemisphere are drawn, but in figs. 107 and 108 all the mitochondria of the cell. Though they are irregularly divided by the maturation divisions their relative amounts in the spermatids do not seem to be greatly different; the evidence is that each long mitochondrial thread becomes divided in the second maturation mitosis if not in the first.

Curious protuberances of the spermatocytes are produced that would seem to indicate pseudopodial movements of the cell body during the maturation divisions. These are shown in figs. 88, 89, 92-95, and since all of these were drawn from cells floating free in the testicular cavity (except fig. 85) these cell processes are not due to the pressure of other cells. Similar protuberances are found upon second spermatocytes (figs. 102, 104, 107); and the left hand one of fig. 104 looks as though it might have persisted since the stage of fig. 94. Frequently mitochondria extend into such protuberances, but not always; therefore they cannot be considered the producers of them.

Towards the close of the second maturation mitosis the mitochondria of each spermatid commence to fuse together (figs. 110, 111), forming an irregular mantle around the connective fibers of the spindle. Then those of each spermatid give rise

to a true 'Nebenkern' (figs. 112, 113), lying at the distal end of the nucleus. This becomes spherical, stains more faintly than before, and differentiates into a central denser zone and a peripheral (frequently vacuolated), more fluid layer (figs. 114-117). This mitochondrial Nebenkern then becomes irregularly pyriform (figs. 118, 120), frequently with a concentric disposition of layers of different densities (fig. 119). Elongating still more it becomes divided into right and left halves (fig. 121), the axial thread lying between the two. One end of the now paired Nebenkern touches the nucleus near the centriole, while the other end grows backward into the tail of the sperm. Figs. 122 to 128 illustrate further stages of the growth of this paired Nebenkern, showing how it keeps pace with the growth of the tail and how its two halves become spirally twisted upon each other. Up into the adult sperm (figs. 127-147) the mitochondrial substance of the Nebenkern is found enclosing the axial thread; but whether it extends the whole length of the tail in the adult sperm was not determined. It becomes gradually more pale in appearance, perhaps due to its becoming thinner in mass. It is certain, however, that this mitochondrial substance extends a long distance into the tail and is not limited to a small middle part; indeed, the adult sperm shows no part comparable with the well defined middle piece of mammalian sperm.

### *E. Discussion*

In cells of the spermatogenetic cycle a number of larger structures are found in the cytoplasm, though generally of less complicated nature than in egg cells because most sperm cells do not appear to produce yolk.

One of these is the mitosome (Platner, '89; Spindelrestkörper, Meves, '97) which arises in the telophase of mitosis from spindle fibers (connective fibers) persisting at the distal end of the cell. It is most prominent in spermatogonia, where it is a dense body, often continuing into a subsequent prophase, and often fusing with mitosomes of neighboring cells. In *Euschistus* a mitosome

is not produced in the spermatocytes or the spermatids. There is much need of a careful study of it in the spermatogonia, for its genesis is by means fully established.

A second body is a more or less spherical, lunar or cup-shaped structure that encloses the centrioles so long as they remain at the distal pole of the nucleus. Meves (1897) has named this the 'idiozome,' and later ('02a, p. 53) the 'centrotheca.' Previously it had been called variously 'sphere,' 'attraction sphere,' 'archoplasm'; but Meves pointed out that since it has no connection with spindle fibers, but disintegrates before the metaphase of mitosis, it is quite different from the attraction sphere of Van Beneden and the archoplasm of Boveri. A new and interesting relation in *Euschistus* is that the idiozome always touches the nucleus at that region where the chromatin composes a particular peripheral chromatin plate; the only other writers who have found a similar chromatin plate are Pantel and de Sinéty ('06), but they described it as a thickening of the nuclear membrane (their fig. 10). In the spermatogonia this chromatin plate, and with it the idiozome, may lie at any pole of the nucleus, though both are always together; but in the spermatocytes the idiozome is invariably placed against the distal pole of the nucleus, where is also invariably the chromatin plate, and in these cells this chromatin plate is composed of the ends of two or three chromosomes. Further, when the idiozome disintegrates, the chromatin plate of the nucleus also becomes disestablished. This constant local connection of these two structures suggests a nuclear influence in the formation of the idiozome. So soon as the centrioles wander apart from each other and pass out of the idiozome, the latter disintegrates; therefore it may be that the centrioles also have something to do with the formation of the idiozome, an idea treated specially by Buchner ('10). In *Euschistus* no idiozome develops in the second spermatocytes or in the spermatids.

In oogonia and the earlier stages of oocytes occur bodies quite comparable to the idiozomes of sperm cells, agreeing in position as well as in containing the centrioles; Van der Stricht ('05, '09)

has shown that in the oocyte of the bat they may persist up to the maturation mitoses.<sup>17</sup> In young oocytes of the guinea pig I have found it to have the appearance described by von Winiwarter.

In animal spermatids there are two cytoplasmic bodies which may be combined or separate, and which have been variously known as *Nebenkerne* and spheres. Meves ('00) has shown that the *Nebenkern* of *La Valette St. George* is composed of granules (*La Valette's* 'cytomicrosomes') which are comparable to the mitochondria of *Benda*, and the true *Nebenkern* is thus mitochondrial. The other 'sphere' of the spermatid Meves ('97, '02) has called an 'idiozome,' homologizing it with the idiozome of a spermatogonium or a spermatocyte. I believe this homology to be a mistaken one, for this sphere of the spermatid behaves quite differently from a true idiozome in being vacuolar and especially in having no connection with the centrioles. Therefore the sphere of the spermatid should no longer be called an idiozome but rather the 'sphere of the spermatid' or the 'spermatidosphere.' In *Euschistus* this sphere of the spermatid, as in a number of insects, undergoes rather complicated movements, then divides into two, and the anterior portion passes to a position at the apex of the sperm and there becomes the lance or perforatorium; it thus produces the same structure as in most other flagellate spermatozoa. But I do not think it likely that this lance is of any mechanical service in penetrating the egg, for in *Euschistus* it is an exceedingly delicate and often branched filament, without any rigidity. Then *Yatsu* ('07) has shown that it is rather the shape of the nucleus of the sperm that is adapted for entering the egg. In *Euschistus* this lance enters into the cytoplasm of a follicular nurse cell; therefore it may rather have the value of a nutritive pseudopodium.

In spermatocytes of *Euschistus* another sphere arises, at about the time of disintegration of the idiozome, and it appears

<sup>17</sup> Compare especially Conklin '02, Van der Stricht '04, '05, '09, von Winiwarter 1900, '09, Bouin, '05.

to have no relation to the idiozome of the spermatocytes nor to the sphere of the spermatids. It has no local connection with the idiozome, the nucleus or the centrioles, and would appear to be a structure entirely distinct from the idiozome, arising in a different place and at a later time; it has only a short persistence and disappears entirely before the maturation divisions. It is not clear whether this body has any homologue in oocytes, for it does not appear related to the vitellogenic layer (that portion of the yolk nucleus not comprising the idiozome).

The other special inclusions of the cytoplasm of the sperm cells are those bodies first recognized by Benda ('98) as specific cell granules, and named by him mitochondria. Henking ('91) was the first to figure them in Hemiptera.<sup>18</sup> Good reviews on these bodies have been written by Meves ('00, '02b, '08b), Benda ('03), Waldeyer ('01), Korschelt and Heider ('02), Goldschmidt ('04). Their great interest lies in the fact that they appear to be constant components of the mature germ cells; and that they develop in embryonic cells into various filar structures such as connective tissue fibrils, myofibrils and neurofibrils, as shown especially by Meves ('07a, '08, '10), Duesberg ('10), Korotneff ('09), Hoven ('10). Interest in them has culminated with the hypothesis of Meves ('08) that they represent an important hereditary substance which has the same relation to the cytoplasm as the chromosomes to the nucleus, and this has received support from the discovery of Duesberg that they persist during cleavage cells in both animals and plants. And it may be noted that Benda's original criterion of them, that they stain blue with his

<sup>18</sup> Their synonymy is already rather confusing. By 'mitochondria' Benda intended granules taking a particular stain, and by 'chondriomita' rows of such granules enclosed in plasma threads. Meves ('07b) proposed the name 'chondriom,' later replaced (Meves, '08) by 'chondriosome,' to include both the single granules or mitochondria proper, and also the chains of such granules; for the latter he proposed ('07b) the name 'chondriokonta,' a chondriokont differing from a chondriomite in being a chain of granules not enclosed in a plasma thread. To corresponding chains Heidenhain (1900) gave the name 'pseudochromosomes,' while Goldschmidt (1904) and some of his followers include the mitochondria under the term 'chromidia.'

alizarine method, has come to be replaced by the criterion of their genetic origin and fate.<sup>19</sup>

Here I propose to discuss the mitochondria merely with relation to their mode of origin, mode of division and history during the spermatogenesis, with reference especially to the phenomena in *Euschistus*. In *Euschistus* the spermatogonia contain a few granules that never stain as intensely as the mitochondria of the spermatocytes, and are never in the form of threads; it was not determined whether these are mitochondria or idiozome remains, but there is more indication of the latter origin. In various other animals, also, they appear to be few or absent in spermatogonia, though described for such cells by Meves ('00, '07), Gérard ('09), Giglio-Tos ('08), Wassilieff ('07), Lams ('09), Dingler ('10), Holmgren ('02), Bouin ('05), Otte ('07) and the Schreiners ('05)<sup>20</sup> They are also few or absent in oogonia. But in *Euschistus* and other objects they are always much more abundant in the growth period of the germ cells, and this is certainly the time of their chief elaboration, a point noted also by Buchner ('10). This is a rather important matter, and easily proven by comparing the large mass of them in spermatocytes with their mass in spermatogonia. The mitochondria thus have their main period of growth and multiplication in the growth period of spermatocytes and oocytes. Their later history, in maturer germ cells, embryonic and tissue cells is in general one of distribution and differentiation.

This leads us to the question of their origin in the growth period. Some writers consider them essentially cytoplasmic structures, with no genetic relations to the nucleus; such are Meves, Duesberg, Bouin, Korotneff, Dingler, while Vejdovský (and his results apply only to oocytes) regards them as portions

<sup>19</sup> Probably many of the bodies described in spermatocytes as yolk globules will prove to be mitochondria, as well as many bodies previously confused with idiozomes. However, Gross has distinguished in *Pyrrhocoris* yolk granules from mitochondria.

<sup>20</sup> The large compact masses in the distal ends of spermatogonia, shown by Giglio-Tos in his fig. 1 and regarded by him as mitochondria, are clearly not such but mitosomes; he was misled by their taking the Benda stain, which shows how little service this stain is as a diagnostic.

of spheres. But it must be said that none of these writers except Vejdovský have paid particular attention to their origin. Pantel and de Sinéty leave the question open, though they show that the 'pseudochromosomes' arise in close contact with the nucleus. Another group of investigators (Dumez, Janssens, Popoff, Wasilieff, Goldschmidt, Buchner, Jörgensen) hold them to be of nuclear origin, for the following reasons: In the pachytene stage of the spermatocytes the chromosomes are frequently, though not in all objects, definitely oriented, radiating in a 'bouquet stage' towards the distal pole of the nucleus, and the idiozome lies at that pole in the cell body; near that pole of the nucleus the mitochondria make their first appearance. This position of the mitochondria, close to a particular pole of the nucleus, is taken to mean that they are produced there by some nuclear activity. Most of these writers maintain that they are engendered by actual emigration of chromatin particles out of the nucleus at that pole, as indicated especially by Janssens, Wasilieff, Jörgensen and Buchner. This idea of chromatin emigration has been particularly instigated by Goldschmidt's view that the mitochondria belong in the class of chromidial formations. In *Euschistus* we found in the resting spermatogonia a particular chromatin plate upon the nuclear membrane, and this is invariably at the point where the idiozome lies; but in these cells there are no demonstrated mitochondria present around the idiozome. In the spermatocytes of this species there is another chromatin plate, here produced by the ends of two or three chromosomes, again always at the pole where the idiozome lies; in the close vicinity of this idiozome the first mitochondrial chains make their appearance. Pantel and de Sinéty found in *Notonecta* that the mitochondria arise between the idiozome and the nucleus, therefore in relation to both. In *Euschistus* it is hard to make sure whether the mitochondria arise from the cytoplasm, from the idiozome or from the nucleus. But the fact is that they originate in the distal pole of the cell, close to the idiozome and the nucleus, therefore it is probable they are produced by either idiozome or nucleus or by a joint action of these. We saw previously that the idiozome probably

stands under the influence of the chromatin plate. In *Euschistus* there is no evidence that the mitochondria are produced by emigrated chromatin particles, and indeed the idiozome intervenes between them and the chromatin plate. Also in this species they certainly have no relation to the idiochromosomes, which may lie upon any point of the nuclear membrane except the idiozome pole and they do not discharge any visible substance into the cytoplasm.<sup>21</sup> Were the mitochondria of strictly cytoplasmic origin it would be difficult to explain why they always arise at a particular pole of the nucleus near the chromatin plate and the idiozome. Therefore it seems a better working hypothesis to conclude that they are produced either by some chemical interaction of idiozome and cytoplasm, or of nucleus and cytoplasm, which would be, in either case, an ultimate nuclear origin. It is interesting to note that their period of early development in the spermatocytes corresponds with the period of conjugation of the chromosomes, and the latter process may be the initial step in their production. This is all in agreement with the concept of the nucleus as the particular formative center of the cell. After the idiozome of *Euschistus* has disintegrated, and the chromatin plate become disestablished, the mitochondria becomes scattered throughout the cell, and then they become larger and more prominent, evidently by autonomous growth. Thus it may well be that the nucleus directly, or acting through the idiozome, gives off ferments in small amounts to the cytoplasm and these ferments in their turn engender the mitochondria that later become self-perpetuating structures.

Another point of interest is how the mitochondria become distributed in the maturation mitoses. In neither *Euschistus* nor other forms is there evidence of autonomous division of them in mitosis; they appear rather to become divided passively by the equatorial constriction of the cell; there is no other mechanism for their division, for they lie outside of the spindle and are

<sup>21</sup> Both Buchner and Wassilieff hold the mitochondria to be produced by a pouring out of substance from the modified chromosomes, though Wassilieff's results on *Blatta* have been contradicted by Morse.

little influenced by the centrioles. In *Euschistus* they lie quite irregularly in the spermatocytes, and generally each thread fails to divide in one of the maturation mitoses; further, it is a matter of chance at what point a mitochondrial thread becomes divided. They become irregularly divided in the two maturation divisions so that varying amounts of them become apportioned to the spermatids.<sup>22</sup> That there is no accurate quartering of the mitochondria in a number of other species results from a study of the figures of various writers, such as the Schreiners ('05, *Myxine*), Meves ('00, *Paludina*, *Pygaera*), Gross ('06, *Pyrrhocoris*), Wassilieff ('07, *Blatta*). But in the bee and hornet (Meves, '07, '08), in *Pamphagus* (Giglio-Tos, '08), in *Blaps* (Benda, '03) and *Stenobothrus* (Gérard, '09) they appear more evenly arranged around the spindles, and in these objects probably become more regularly divided. But there is good evidence that in certain cases it is a matter of chance how they become divided, in which regard they differ markedly from the chromosomes.

The mitochondria in the spermatid of *Euschistus* coalesce to produce the true *Nebenkern*, which elongates and forms a pair of narrow bands lying on the sides of the axial thread and extending from the head probably to the end of the tail of the spermatozoon. A similar metamorphosis of the *Nebenkern* is known for flagellate spermatozoa in a number of invertebrates, while in the mammals the mitochondria engender a spiral filament around the middle piece. There is now, however, considerable evidence that in many forms of flagellate spermatozoa, such as those of mammals and molluscs, the whole tail enters the egg in fertilization, and is not left outside the egg (contrary to earlier observations); therefore it is probable all the mitochondrial substance of the sperm enters the egg; much more substance, accordingly, than merely the chromatin of the head.

Since then fertilization brings together the mitochondria of two parents, it now becomes of great importance to trace

<sup>22</sup> I have previously intimated, ('10b) the possibility that the relative amount of the mitochondrial substance received might determine the sex-preponderance character of a sperm, a matter unfortunately very difficult to test.

the history of the mitochondria during the fertilization of the egg, and especially the behavior of those furnished by the sperm.

PREFORMATION AND EPIGENESIS IN THE GERMINAL CYCLE, AND  
SEGREGATION OF THE GERM CELLS IN ONTOGENY

The discussion of embryologists upon preformation and epigenesis has treated mainly the phenomena of somatic differentiation, the factors regulating the growth of the embryo from the egg. If it be not too premature to speak of a consensus of opinion reached in this discussion, it would be to the effect that epigenesis and preformation are not mutually exclusive, but that both probably proceed at the same time.

Here it is my wish to call attention to the fact that in the history of the germ cells may be recognized both preformation and epigenesis, whereby the germinal cycles offer a certain parallel to the somatic.

In the spermatogenetic cycle a number of spermatogonial generations occur, during which the number of the chromosomes remains constant and no marked cytoplasmic specializations take place. But in the spermatocytes remarkable differences arise suddenly: the chromosomes group themselves into gemini, the mitochondria increase rapidly in amount, the whole cell becomes larger, and frequently the centrioles take on unusual forms and positions (as upon the cell membrane). Somewhat similar changes occur in the oocytes, and more complex ones, owing to the production of yolk substance. The spermatids often undergo a marked metamorphosis. As one reviews the sum total of these processes the conviction arises that there are here in the completest form both preformation and epigenesis. The chromosomes are on the whole the most stable parts, apparently continuous from generation to generation, and though they may pass through marked changes in forming the sperm nucleus, they later emerge, in fertilization, under the same forms that they had previously. On the whole they seem to be the particular preformed bodies of the germ cells. But this is not the case with certain other cell constituents. For in *Euschistus*, which seems to exemplify in main features the sperma-

togenetic relations of most insects, a true idiozome arises in the spermatocytes, mitochondria develop around it, the idiozome disintegrates and a sphere appears, this sphere disappears entirely and in the spermatid another sphere is produced that originates another new body, the perforatorium.

It is clear that the first spermatocytes and oocytes are the most interesting cells of their respective cycles, for they exhibit the most significant processes—conjugation of chromosomes, reduction division, elaboration of mitochondria. In them the history of the cytoplasmic parts is markedly epigenetic. And another series of epigenetic changes is exhibited by the histogenesis of the spermatozoon.

There is then a parallel with the somatic cycle, which begins with an undifferentiated and terminates with a highly differentiated condition. The ripe ovum and spermatozoon are much more differentiated than the spermatogonium or oogonium; each of them enters, with the commencement of the growth period, upon its period of specialization.

The recognition of this resemblance may throw light upon the problem of the segregation of the germ cells. By what process is it that certain cells of the embryo are held back from somatic differentiation to become germ cells? In other words, why do not all the cells become specialized? The answer, it seems to me, is to be sought in the distribution of the specializations of the fertilized egg to the cells of the embryo. One set of specialized structures of the germ cells, the mitochondria, are now known to give rise to various important specializations of body cells. The mitochondria, that are elaborated, in greatest part at least, during the growth period of the germ cells, persist from the fertilized egg into cleavage stages, and ultimately transform into various specialized fibrillar structures. With this in mind the setting aside of germ cells from body cells could be explained mechanically as follows: any cleavage cell which failed to receive mitochondria, or failed to receive particular ones or a particular amount of them, would be incapacitated from engendering such somatic specializations, it would thereby become a germ cell. This might appear contrary to the idea

that the body cells become different from the germ cells by a mechanical process of chromatin diminution, as in *Ascaris*. But this is quite conformable to our argument, for in *Ascaris* those cells which become body cells are the ones that include the cast-off chromosome ends in their cytoplasm, and it will probably be found that these ejected chromosome parts engender such cytoplasmic differentiations as characterize the body cells.

Either this mechanism of the segregation of the germ cells may be admitted, or else one that would cause the mitochondria of prospective germ cells to remain unaltered or latent. Fortunately this is a matter that may be tested by observation. For if the first supposition be correct we should find that during cleavage an unequal distribution of the mitochondria occurs, just as we know occurs in the case of yolk granules. If the second be correct, then while the mitochondria are undergoing developmental changes in some of the cells, they should be found to remain relatively unaltered in others—the prospective germ cells.

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## PLATES

All the figures have been drawn to the same scale with the aid of the camera lucida at the level of the base of the microscope, with the Zeiss apochromatic immersion objective 1.5 mm., and ocular 12; the original dimensions have been reduced one-fifth.

The following abbreviations have been employed:

c. centriole	m. minute chromosome
ch. c. chromatoid corpuseles	mit. mitosome (spindle remains)
D. larger idiochromosome	pf. perforatorium
d. smaller chromosomes	pl. plasmosome
id. idiozome	sp. sphere

## PLATE 1

### EXPLANATION OF FIGURES

1 and 3 from testis no. 103, all others from testis no. 282.

1 Penultimate spermatogonium, rest; (follicle 5).

2 The same, pole view of equatorial plate; (follicle 6).

3 Portion of a rosette of ultimate spermatogonia; (follicle 6).

4 Anaphase of ultimate spermatogonium; (follicle 4).

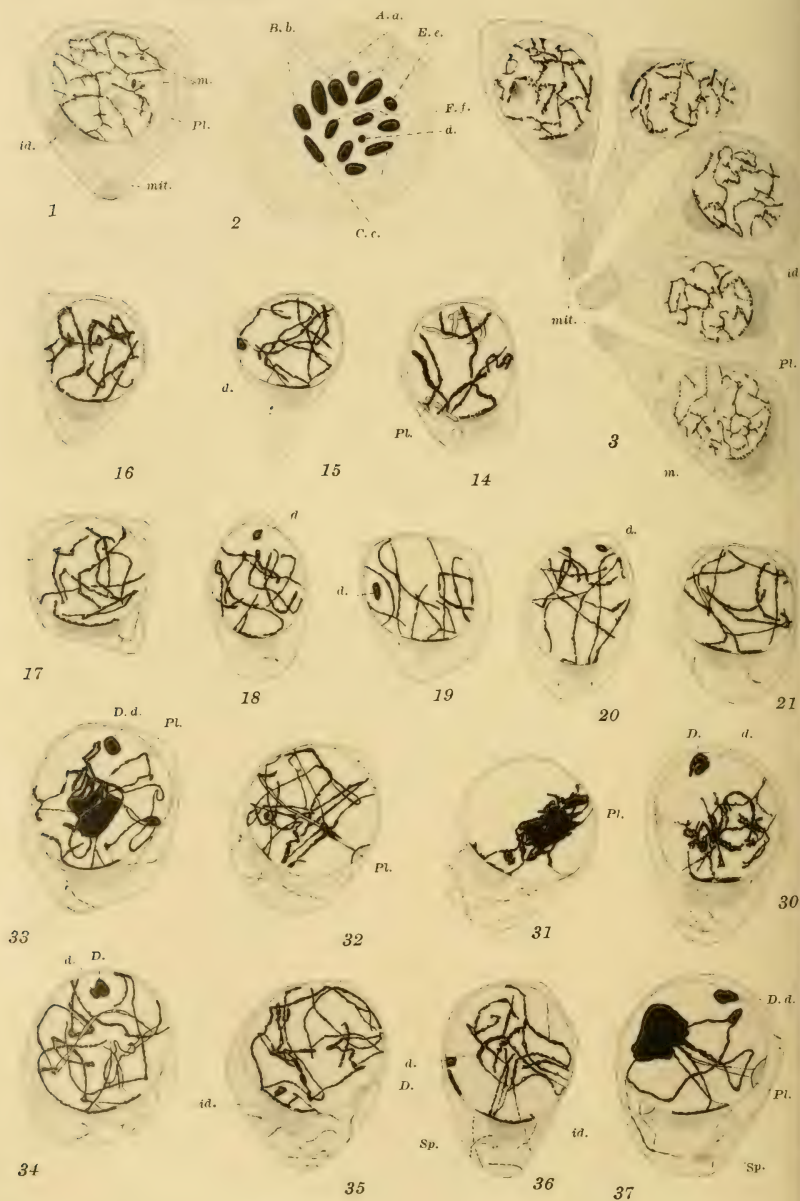
5 Later anaphase of the same; (follicle 4).

6-41 Successive stages of the early growth period of the first spermatocytes.

All the cells from follicle 4 except the following: Figs. 23, 24 from follicle 5; figs. 10, 11, 15, 22 from follicle 6.

# SPERMATOGENESIS OF AN HEMIPTERON

T. H. MONTGOMERY







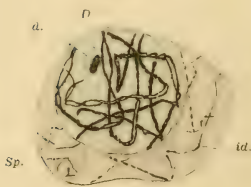
## PLATE 2

### EXPLANATION OF FIGURES

Successive stages of first spermatocytes, all from testis no. 282; all from follicle 4, except fig. 49 from follicle 5.

## SPERMATOGENESIS OF AN HEMIPTERON

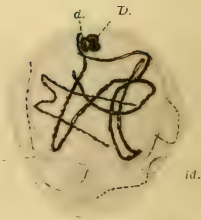
T. H. MONTGOMERY



42



43



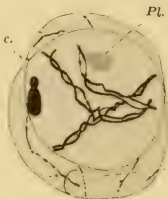
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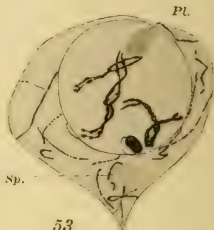
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55



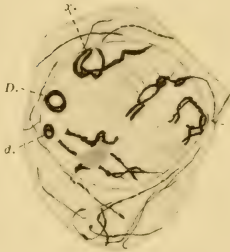
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53



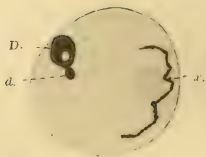
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58



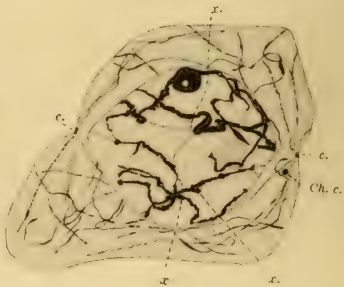
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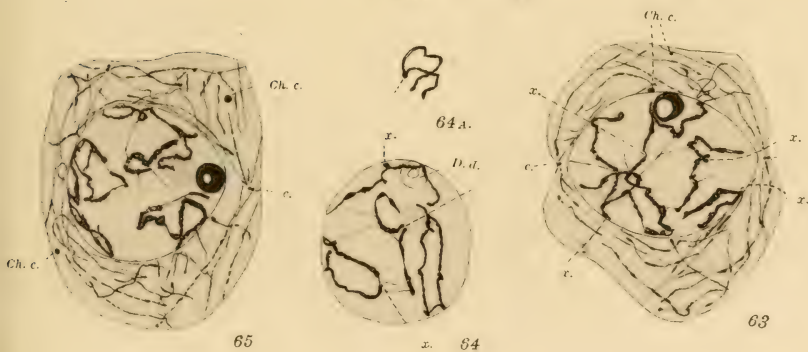
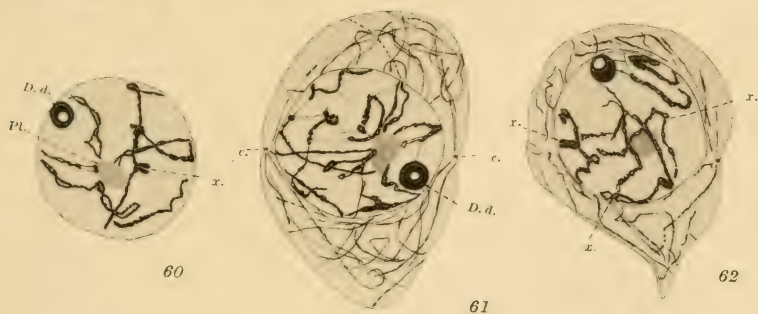
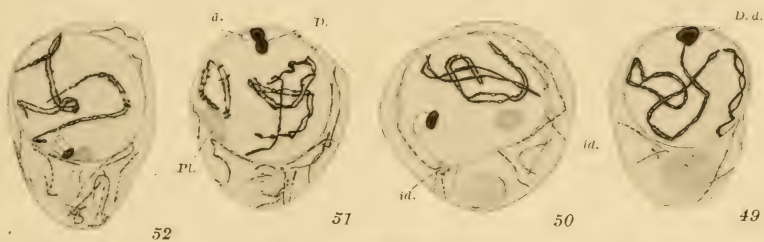
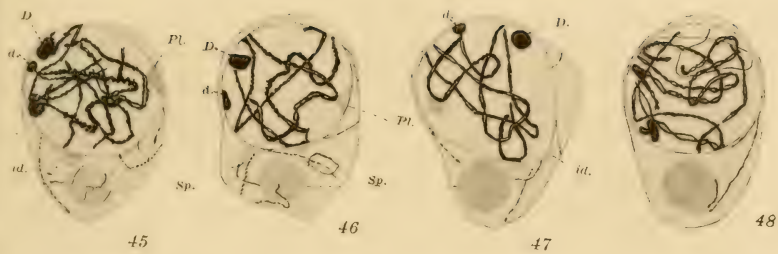
68



67



66





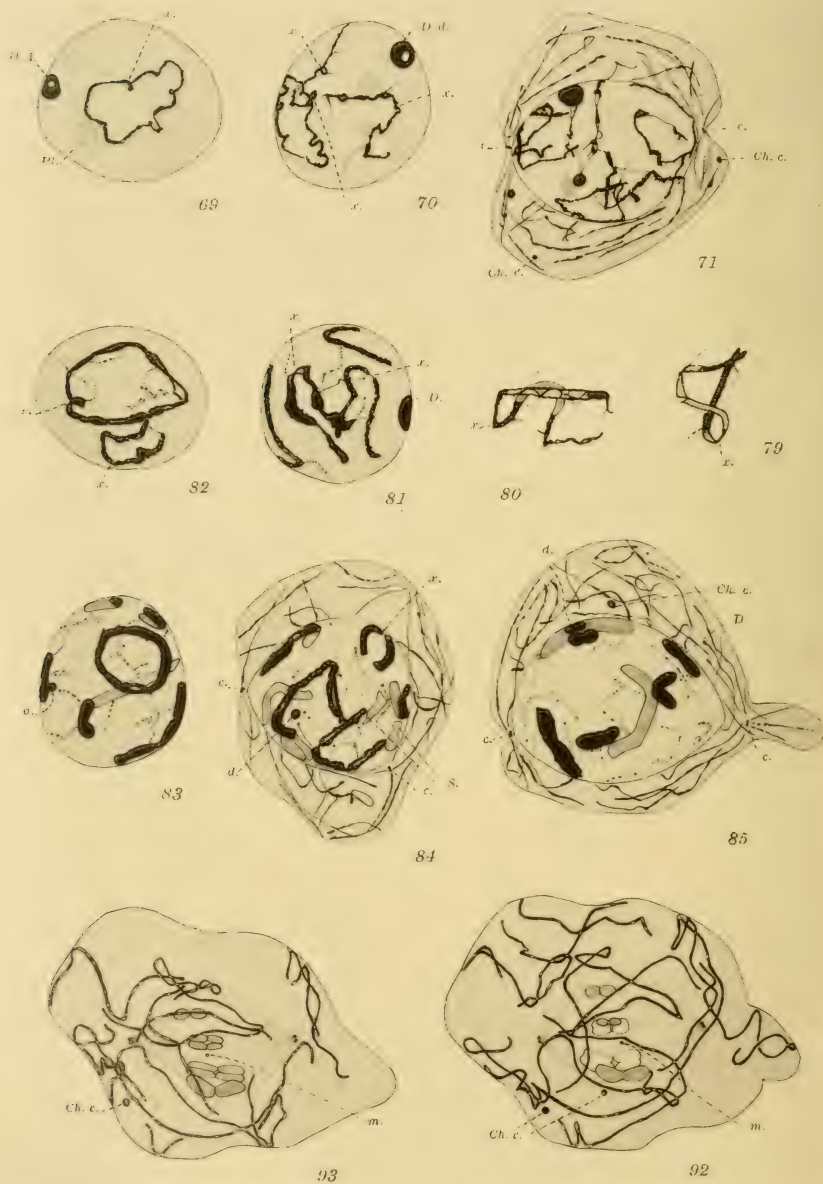
### PLATE 3

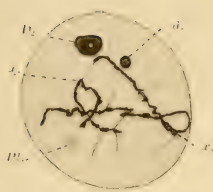
#### EXPLANATION OF FIGURES

Successive stages of first spermatocytes, figs. 74, 75, 82, 83 from testis, no. 286; 86-93 from testis no. 265; the remaining figures from testis no. 282. Figs. 74-83, 86-89, 91-93 from follicle 6, the remaining from follicle 4.

69-86 Later prophases of the first maturation spindle.

87-93 First maturation spindle.

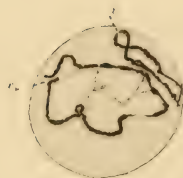




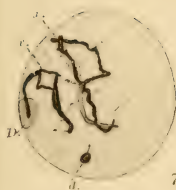
72



73



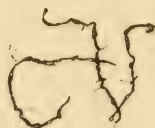
74



78



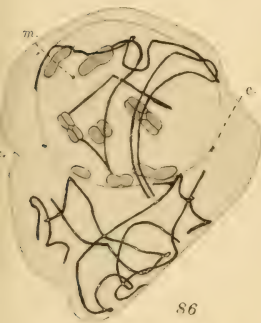
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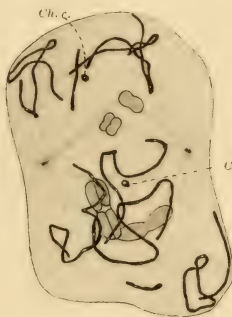
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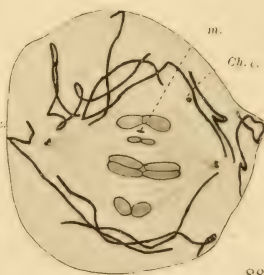
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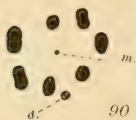
86



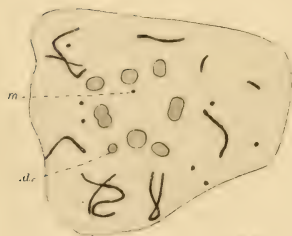
87



88



90



91



89



## PLATE 4

### EXPLANATION OF FIGURES

111 from testis no. 282, the others from testis no. 265. All the figures from follicle 6 except the following: 98 and 100 from follicle 4; 95-97, 110 from follicle 5.

94 Metaphase of first maturation.

95-100 Anaphases of first maturation, fig. 98 being a polar view of a daughter chromosomal plate.

101 Polar view of metaphase of second maturation.

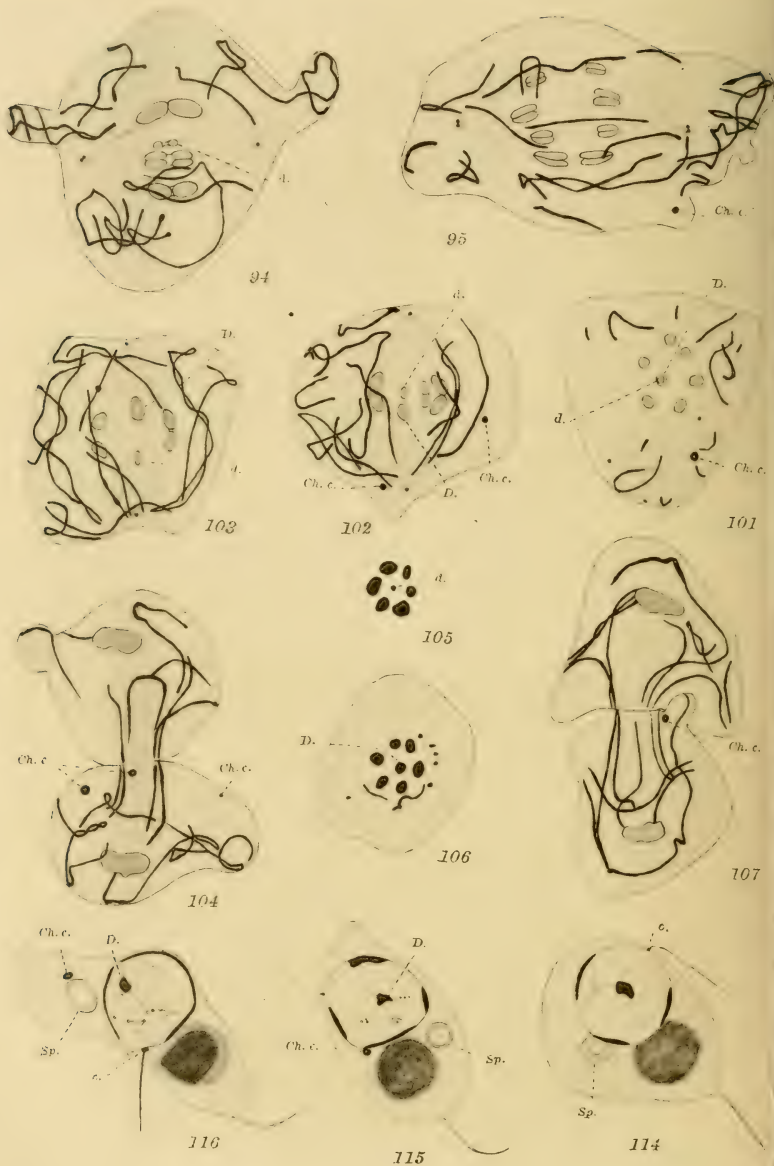
102-104 Lateral views of second maturation spindles.

105-106 Polar views of daughter chromosomal plates of the second maturation spindle.

107-111 Lateral views of later second maturation spindles.

112 An entire spermatid (on the right) nearly completely separated from its sister (shown in part outline on the left).

113-116 Earliest stages in the histogenesis of the sperm.





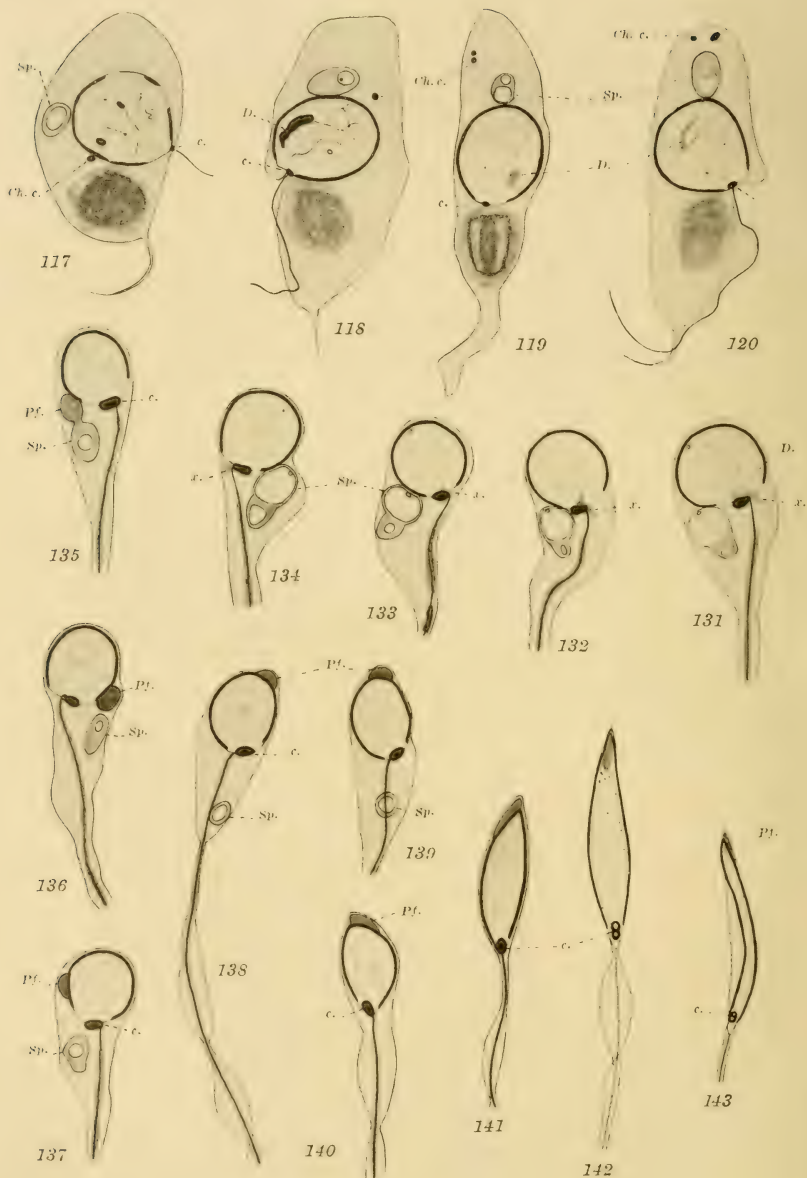


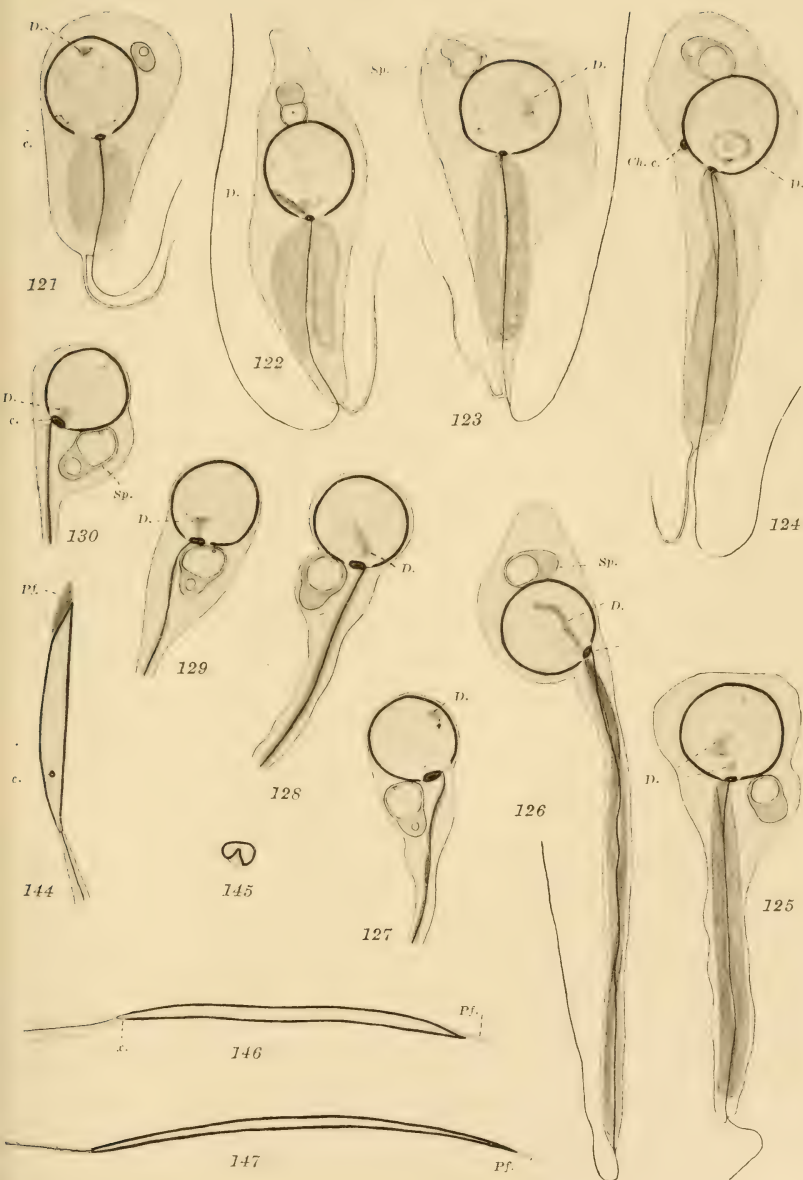
## PLATE 5

### EXPLANATION OF FIGURES

Histogenesis of the sperm. All the figures represent lateral views, except 145, which illustrates a transection of a sperm bead of the stage of fig. 144; the entire spermatozoon is shown in 117-126, in the others only the head and the proximal part of the tail are represented.

All the figures are of cells from follicle 6; 117-122 are from testis no. 265; 135-137 from testis no. 116; the remainder from testis no. 282.







# THE LIFE HISTORY OF THE SCOLEX POLYMORPHUS OF THE WOODS HOLE REGION

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*From the Zoological Laboratory, University of Missouri*

THIRTEEN FIGURES

## CONTENTS

Introduction.....	819
Methods.....	820
The nature of the Scolex polymorphus.....	821
The normal occurrence of parasites in sand sharks taken at random.....	827
The infection of sand sharks with the Scolex polymorphus.....	829
Summary.....	849
Literature cited.....	848

## INTRODUCTION

In attempting to determine the life cycle of *Crossobothrium laciniatum*, a cestode found in the sand shark, (*Carcharias litoralis*) of the Woods Hole region, attention was at once directed to the cestode larva known as the *Scolex polymorphus*, which seemed not unlikely to be the young of this species. The studies here described were begun in the hope that these two forms would prove to be a single species, since both are admirable for laboratory purposes and have been so used by students at the Marine Biological Laboratory for many years. It appears, however, that such a relationship does not exist; for the results of the experiments give strong, though perhaps not entirely conclusive, evidence that the *Scolex polymorphus* develops into the species *Phoreiobothrium triloculatum* (Linton) and show conclusively that it cannot be the young of *Crossobothrium laciniatum*.

The material was collected and the experiments performed during the summers of 1903 and 1904 at the Marine Biological Laboratory and the United States Fisheries Bureau Laboratory at Woods Hole, Mass., and I am indebted to those in charge of these institutions for substantial aid in the prosecution of the work.

#### METHODS

The sharks used for infection and the squeteague from which the specimens of the *Scolex polymorphus* were obtained, were all taken during the summer months in the fish traps near Woods Hole. The sharks were marked by means of numbered copper tags, fastened to the dorsal fin and were kept in wooden fish cars about 5 x 6 x 14 feet, several of which were fastened together to form a float upon which much of the work could be conveniently carried on. At the outset it was necessary to devise some apparatus by which the animals could be held securely in a position convenient for any necessary operation and which could be manipulated with safety by a single person. The holder which was finally constructed consisted of a trough about four and one-half feet in length, formed by nailing two boards together and across their ends two shorter strips after the fashion of a farmer's 'pig-trough.' The top of this trough was covered by a hinged lid which, when fastened down, left the head and tail exposed but held the body of the fish securely along the greater part of its length. The cross piece of one end was hinged to the surface of the float where the holder was being used and to the free end was hinged a support, which, when swung out, held the contrivance at any desired height as the free end was lifted toward the upright position. By working rapidly, it was possible, with the fish thus held securely, to complete the necessary treatment in a few moments, and any more elaborate apparatus providing for the irrigation of the gills seemed unnecessary, since the sharks gave no indication that their vitality had been impaired by this brief exposure to the atmosphere. In operation this holder was safe and in every way satisfactory. The shark was dipped up with

a hand net and placed in the trough ventral side uppermost. Upon fastening its lid, the holder was at once raised to a convenient position and the operator could then quickly introduce any desired object into the mouth cavity. In feeding, the jaws were pried open, a piece of food placed in the mouth and after being guided past the gill arches was thrust down the oesophagus by means of a billet of wood. The oil of male fern and the calomel, used in the attempts to rid the sharks of their previous infection, were given in gelatin capsules having a capacity of 2 cc. These were placed in a piece of galvanized iron pipe, which had been pushed down the oesophagus until it reached the stomach, and the capsule forced down the pipe with a small wooden rammer. This proceeding, or the giving of food, could be accomplished with comparative rapidity and the sharks were often back in the car within two or three minutes after being taken out. There was no sign of the regurgitation of food or drugs, though the bottoms of the cars were carefully inspected during the hours just after treatment, and both food and drugs were found in the digestive tracts of specimens which happened to be examined during the first few days. There can, therefore, be no doubt that both remained in the stomach when once introduced. Further details of methods used are given at the appropriate places throughout the paper.

#### THE NATURE OF THE SCOLEX POLYMORPHUS

Before describing the experiments in which the larval cestode known as the *Scolex polymorphus* was used to infect the sand shark, it may be well to offer a word of explanation regarding this form. The name *S. polymorphus* has been applied by Linton to a cestode larva found in a considerable number of fishes from the Wood Hole region. While most abundant in the squeteague (*Cynoscion regalis*) and the common flounder (*Paralichthys dentatus*), he has found it in varying numbers in some twenty-eight other teleosts, the list of which is given on page 413 of his 1901 report. This larva, which I have represented in figs. 1, 2, 3, 4, 12 and 13 of this paper, is frequently met with in a stage slightly

younger than the one here shown. In such a young condition it lives in the intestine of its teleost host, moving freely about and attaching itself by means of its four suckers. The slightly older stage which these figures represent and which was used in my experiments, is found in the cystic duct of some of these fishes, notably the squeteague and the common flounder. The specimens which I used for infection purposes all came from the first of these two hosts.

The name *Scolex polymorphus* originated with Rudolphi ('08) and has since been frequently applied to such larval forms taken from many different fishes, and by Van Beneden ('50) from crabs of the genera *Carcinus* and *Eupagurus*. Zschokke ('86), discussing such forms, showed that the mono-, bi- and triloculate types of bothria (figs. 2, 12 and 13 of the present paper), which can be discovered when any considerable number of individuals are examined, are only developmental stages of the same form, and his results indicated that the *S. polymorphus* with which he worked was the young of the genus *Calliobothrium*, and not of *Onchobothrium* as had been previously suggested. These authors of course studied specimens from European waters.

Monticelli ('88) in an extensive paper upon the *S. polymorphus* of the region about Naples, showed that the larvae, which he found in a large number of fishes, though most common in the flounders, were the young of *Calliobothrium filicolle*. This conclusion was based upon the close anatomical resemblances between the more advanced larvae and young specimens of *C. filicolle* and upon experiments in which a species of *Torpedo*, after being freed of all parasites by starving (a method which his experience and that of the collectors at the Naples station had shown to be effective), was then fed for a time upon specimens of *Arnoglossus* known to contain the *S. polymorphus*. As a result of this, young specimens of the *C. filicolle* were obtained from the torpedoes so treated, and this taken with the anatomical resemblances seemed conclusive evidence. Monticelli has reviewed the literature exhaustively and he gives (p. 89) a list of thirty-six cases in which authors have appended various specific names to the term *scolex* and made as many different species

of this single form. He refers to these names as synonyms and apparently considers all the forms from whatever host to be the young of a single species.

Although my experiments indicate that the *S. polymorphus* of the Woods Hole region develops into *Phoreiobothrium triloculatum* and not into a species of *Calliobothrium*, a comparison of the larvae, as taken from the fishes about Woods Hole, with the description which Monticelli gives shows that the two types are closely similar, probably almost indistinguishable. The myzorrhynchus, bothria with one, two or three loculi, two faint red pigment spots in the neck region of some of the specimens, the general shape of the body and the characteristic movements, are apparently identical. Only in the case of the hosts they inhabit is there any very apparent difference, which is of course necessitated by the differences in the piscine faunas of two such widely separated regions.

In his paper published in 1897, Linton suggested that the *S. polymorphus* perhaps represents the young of a number of different cestodes, and also that none of the fishes (teleosts) in which he has found it is the true host of either larva or adult. He considers such larvae when found in teleosts to be 'xencsites,' or misplaced parasites, and thinks that the true intermediate hosts may be found among the species of crabs which frequent the feeding grounds of these fishes. In support of this view, he cites the fact that the existence of similar larvae in crustacea has been recorded by Van Beneden ('59). Should this hypothesis prove correct, we should have a case where the transfer from such a host to a teleost fish, while not fatal to the parasite, still presents conditions under which it can develop but a little way beyond the stage already attained. Basing his conclusion largely upon the presence of a median proboscis-like structure (the myzorrhynchus) at the anterior end between the bothria, Linton ('97) expressed the opinion that our *Scolex polymorphus* is the young of the genus *Echeneibothrium*. This is not, however, as strong a clue as might seem, for in the adult *Calliobothrium filicollis*, to which Monticelli's larvae developed, this structure is quite degenerate, though well marked in the larva, while in the adult

of *Calliobothrium leucartii* and *Calliobothrium verticillatum* there is no trace of such a structure.

In a later paper on the parasites from the fishes of Beaufort, North Carolina, Linton ('04) finds the *Scolex polymorphus* in many of the fish examined and speaks of the larvae as follows (p. 326):

The larval cestodes, doubtless representing several genera, recorded in Parasites of the Woods Hole Region under the name of *Scolex polymorphus*, were found in thirty-four of the fifty-nine Beaufort fishes examined. As at Woods Hole these forms are found not only in the alimentary canals of their hosts but also in the cystic ducts of several. They are almost never absent from the cystic duct of *Cynoscion regalis*. In all cases, where these worms have been obtained from the cystic duct and from the intestine of the same fish, those coming from the cystic duct are larger, plumper, and more opaque than those from the intestine. Some of the older larvae suggest the genera *Calliobothrium*, *Acanthobothrium* and *Phoreiobothrium*.

Again, in speaking of the parasites of the sharp-nosed shark, *Scoliodon terreae-novae*, under *Phoreiobothrium triloculatum*, he says (p. 343):

1 scolex, no segments yet developed; length 2 mm.; hooks small. This specimen looks very much like some of the more advanced specimens of *Scolex polymorphus* which have occasionally been found, save that the bothria have assumed the characteristics of *P. triloculatum*.

On page 359, under the parasites of the pipe fish, *Siphostoma fuscum*, he notes that the specimens of the *Scolex polymorphus* had "bothria with two costae and rudiment at anterior end, suggesting loculi which occur at the anterior end of bothria in *Echeneibothrium* and *Acanthobothrium*; no red pigment."

On page 407 under the parasites of the toad fish, *Opsanus tau*, he speaks of a specimen which was "probably a young *Calliobothrium*," and of another which "had the characteristic bothria of *Echeneibothrium* and *Rhinebothrium*. Its prominent muscular proboscis, (myzorhynchus), if retained in the adult would place it in the former genus." Again, in another lot, "The largest had bothria which resembled those of *Calliobothrium* and *Acan-*

thobothrium, but without hooks." And finally, others which had "red pigment, two costae, one specimen noted with rudimentary hooks (*Calliobothrium* or *Acanthobothrium*)" and in another lot a specimen is recorded with the "rudimentary hooks and pigment spots."

Under the parasites of the sole, *Symphurus plagusia*, there are mentioned specimens which are "comparatively large, with two costae and red pigment like young *Acanthobothrium*, but without hooks."

Fig. 80, plate 12, shows a young specimen of *Calliobothrium* with rudimentary hooks, but otherwise much like the *Scolex polymorphus*.

In view of these later observations of Linton and Monticelli's results, one would expect the *Scolex polymorphus* from about Woods Hole to develop into one of the species of *Calliobothrium* found in this region, or perhaps some other species of the family *Onchobothriidae*, and this last is what I believe happens in the case of the larvae with which my experiments were performed. Since two species of the genus *Calliobothrium* (*C. verticilatum* and *C. eschrichtii*) have been found in our region, by Linton ('99) who records this species from the dogfish (*Mustelus canis*), and since a considerable number of species belonging to most of the genera of the family *Onchobothriidae* have been described from Woods Hole by Linton, it would seem not at all unlikely that experiments made by feeding the *Scolex polymorphus* from the various teleosts to skates, dogfish and sharks might connect these larvae with other genera of the *Onchobothriidae*. Such experiments would be likely to give precise evidence for or against Linton's belief that the larvae represent the young of more than one form and they might give us data for further consideration of the whole question of xenositis, which Linton suggests is the condition of these larvae when found in teleosts.

In considering the possibility that the various forms of crustacea are the true intermediate hosts in which the development was begun, I have made a careful tabulation of the food of these fishes as recorded mainly by Linton ('99), but also in more detail for a smaller number of fishes in the work of Peck ('95). This

tabulation is not given since it is merely a compilation and the important point ascertained can be briefly stated; namely that crustacea of various sorts, shrimps, amphipods, isopods, crabs, etc., are an important food with almost all these fish. In cases like the squeteague and blue-fish, where they are not so important an item, it is noticeable that various crustacea-eating fish are a common food. This would account for the great numbers of the *Scolex* in the squeteague, which would then be like a sieve in which were retained many larvae which had come originally from crustacea through the medium of another fish. The flounder, *Paralichthys dentatus*, is the other fish in which Linton has found the *Scolex* most abundant. Its food consists of smaller fish and a large proportion of various crustacea, so that in this case the *S. polymorphus* might be obtained directly from crustacea, or indirectly from another fish. I have also tabulated the food of the fishes from which Linton has *not* recorded the *Scolex* to see whether crustacea form as large an element in their food supply, but no satisfactory facts can be gathered for the reason that the list of those containing the larva comprises the greater number of our smaller and more common fishes and because, as Linton expressly states, no systematic search has been made and hence the fact that the larvae have not been recorded from any fish may have little importance.

I quite agree with Linton's suggestion that this widely distributed larva, though it does not resolve itself into several easily recognizable types in the larval condition, may eventually be shown to represent the young of more than one cestode, and if I am correct in my conclusion that the *S. polymorphus* with which my experiments were made develops into *Phoreiobothrium trilocolatum*, whereas the form with which Monticelli worked is the young of *Calliobothrium filicolle*, this may be the beginning of evidence which will give Linton's interpretation a secure foundation and thus the name 'polymorphus,' which seems originally to have been given because an individual larva of this sort can assume such diversity of shape, may come to have a new significance from the existence of many species under a guise which does not show differences by which each may be recognized.

The close resemblance of our *S. polymorphus* to the forms upon which Monticelli (op. cit.) worked, makes a discussion of the anatomy superfluous beyond what is shown by my figures and their explanations which have been made quite full. The differences are only of a minor nature and hence this author's account is adequate for the anatomy of our forms.

#### THE NORMAL OCCURRENCE OF PARASITES IN SAND SHARKS TAKEN AT RANDOM

A knowledge of the normal content of parasites found in the sharks, as collected, was important both for its bearing upon the results of treatment which attempted to rid them of all parasites, and upon the results of any artificial infection with young cestodes. There are very few sand sharks examined which have not some infection with *Crossobothrium laciniatum*, which is the only cestode parasite known to infect the digestive tract of this host in considerable numbers. Some records by Linton ('99, p. 429) are here tabulated as quite representative of any dozen specimens taken at random.

*Table from Linton's records*

DATE	NUMBER OF SHARKS EXAMINED	NUMBER OF <i>C.</i> <i>LACINIATUM</i>
July 17, 1899.....	1	20
July 21, 1899.....	1	several
August 9, 1899.....	1	numerous
August 12, 1899.....	1	2
August 15, 1899.....	1	1
August 17, 1899.....	1	4
August 18, 1899.....	1	55
August 19, 1899.....	1	12
July 20, 1900.....	1	47
July 20, 1900.....	1	16
August 12, 1900.....	1	numerous
August 13, 1900.....	1	106

From my own records, the results are similar, though the counts are usually higher because a careful search was being made for the small young specimens. The following table is from six sharks examined in 1904.

DATE	SHARKS	C. LACINIATUM	
		Adult	Young
July 28.....	1	34	50
July 30.....	1	20	10
August 1.....	1	30	6
August 3.....	1	30	50
August 4.....	1	35	25
August 5.....	1	50	30

During the years 1899-1910 this parasite has been used for study by the students in one of the courses given at the Marine Biological Laboratory at Woods Hole and we have always been able to obtain an abundant supply when several sharks were available. Sometimes the first shark opened has yielded all the material needed and it has never been necessary to examine more than four or five. Most of the actual counts recorded in my notes in 1903-04 were made upon sharks in which search was being made for specimens showing the early stages of proglottid formation and for this reason the sharks recorded are perhaps those which seemed, when first opened, to have an abundance of the parasites. Linton's records as given in the first table are therefore more fairly representative.

As a further example of their abundance, my notes record the examination on August 11, 1904, of ten sand sharks, taken in the traps on that date. Every one of the ten was infected and in only two cases was the number of the parasites noticeably small. Count was not made because it was evident that the amount of infection averaged substantially the same as that shown by Linton's record.

From these data it is evident that one rarely finds a sand shark which has not some infection; and from the fact that the worms may be found in all stages of development, from the specimens just beginning to form segments to the large adults which are shedding motile proglottids, one may conclude that the source of the infection has been in contact with the sharks within a quite recent period, if, indeed, it is not acting upon them throughout the summer.

THE INFECTION OF SAND SHARKS WITH THE SCOLEX  
POLYMORPHUS

The first attempt at infection of the sand sharks with the *Scolex polymorphus* was made with fish which had been held without food for a period of three weeks, a treatment which Monticelli ('88) found effective in ridding a species of *Torpedo* of its *Calliobothrium*. In all, eleven fish were so treated and each was then given all the larvae obtainable from twelve squeteague, a dose which was estimated at not less than five hundred larvae for each shark. Each fish was fed at the time of the infection and in the three weeks after infection, during which they remained alive, each was fed four times. For food, the flesh of the squeteague was used, an amount about equal to one-third, or one-half the bulk of a good sized fish being used at a feeding; my guide in this matter being the size of the pieces of food commonly found in the stomachs of recently captured sharks, which had fed under natural conditions. Judging from the rate of digestion, as observed on several occasions, this amount of food was unnecessarily large. Such an amount once a week would be ample for sharks in captivity. Moreover, the choice of food was not good; for one may be introducing almost any kind of a cestode larva by feeding the flesh of a teleost fish. In using for infection sharks which had not received any treatment, other than the three weeks' starving above mentioned, I was, of course, aware that one might expect each one of them to contain the normal infection of *C. laciniatum*. It seemed, however, that if the *S. polymorphus* from the squeteague did represent the larval form of *Crossobothrium*, it would develop readily in its normal host, and that the introduction of a very large amount of infection would perhaps give the fish thus treated so many young worms all the same size, as to show that they could only have come from the larvae introduced by the experimental infection.

Three weeks after the infection these eleven sharks were killed and their digestive tracts carefully examined. Each contained adult specimens of *C. laciniatum* in numbers sufficient to indicate that all the sharks had their normal complement of parasites and

therefore that the three weeks without food had produced no effect. In eight of the fish there were a considerable number of young specimens of *C. laciniatum* in all stages of proglottid formation, but as similar stages are commonly found in all sharks (Curtis, '03 and '06), their occurrence here was no evidence that they had come from the introduced *Scolex polymorphus*, and the diversity of their stages made any such interpretation out of the question. In these eight specimens there were found in addition to the young and adult *C. laciniatum*, an unusual number of individuals of the species *Crossobothrium angustum*, which Linton ('99) p. 426), records as a frequent parasite of the dusky shark, *Carcharinus obscurus*, and the blue shark, *C. milberti*. Although present in greater numbers than I have found in any other lot of sand sharks, these *C. angustum* were of all stages from young to adult and there seemed, therefore, no evidence which would connect them with the larvae which had been introduced by my infection. In the whole number of sharks I found upwards of fifty young of another cestode, all in about the same stage, and with well developed scolices and the segmentation into proglottids just beginning. Because of their conspicuous and characteristic bothria, these were at once recognizable as the young of the species *Phoreiobothrium triloculatum*, a form which Linton has described from the dusky shark and which is represented in figs. 5, 6, 9, 10 and 11 of this paper. Unfortunately, my records give only the fact that each of these sharks contained some *Phoreiobothria* and fail to give their distribution in the individual sharks.

The occurrence of this species in the sharks of this experiment would indicate, when taken alone, hardly more than that *P. triloculatum* is sometimes found in the sand shark, even though the only sand sharks in which I have found it are the ones previously infected with the *Scolex polymorphus*. The fact that the individuals were all of about the same early stage is more important, though not much stress can be laid upon it because of the failure of my records to state the distribution of these worms in the individual fish. I regard the results of this attempt at infection, which was the only one I was able to carry through in the

first summer of my work, as valuable only because they support the more satisfactory results obtained in the work of the following summer. A further discussion is, therefore, deferred until the results of the later work have been presented.

Having been unsuccessful in the attempt to reduce the number of parasites by the process of starving, for as long a time as was available, if the sharks were to be used for any subsequent experiments, attention was directed at the beginning of my second summer's work to the discovery of an effective method by which the sharks could be entirely freed of their cestode parasites. For this, I used the oil of male fern, one of the most powerful vermifuges used in human and veterinary practice. Following this practice, the dose of the oil was followed after an interval of from twenty-four to forty-eight hours by one of calomel. The manner of introducing these drugs into the stomach of a shark is explained in the earlier section of this paper where the general methods of work are given. In the tables upon the pages which follow will be found the detailed results obtained with the several lots of sharks treated in this manner. The necessary general explanations will be given in the discussion of the first table, while in the discussion of the subsequent tables I shall give only the facts which the tables show.

TABLE 1

*5 sharks, captured before July 2, kept without food until July 22*

July 22, all sharks given 2 cc. each of oil of male fern

July 23 the four surviving sharks given 1 cc. of calomel

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
July 23. ....	No. 1	Dying	6 <i>C. lacinia-</i> tum found dead	
July 26. ....	Nos. 2, 3, 4, and 5	Killed	No signs of cestodes	Mucous mem- normal

Table 1 shows the results obtained with five sharks which were starved from three to four weeks after capture and then given

2 cc. of oil of male fern, followed twenty-four hours later by about 1 cc. (measured dry) of calomel. The dates and other points of importance are shown in the several columns, under appropriate headings. For convenience of reference, each shark is given a number. The column which shows the 'condition at death' is inserted because specimens did not always survive the treatment and thus some of those examined had died from its effects. Such specimens are marked 'dead' while others which were killed because they seemed about to die are marked 'dying.' The specimens which are marked 'killed' are those which appeared to be in perfect condition when they were taken and killed for examination. The specimens found soon after death ('dead') and those which were killed when they seemed likely to die ('dying'), presented data of some value, for the reason that under normal conditions the death of the shark is not followed at once by the death and consequent disintegration of the parasites. One finds that living cestodes can be obtained from untreated sharks, which have been dead in the water, or lying exposed to the air upon the wharf for five or six hours and I have often seen, in my work with the students at the Marine Biological Laboratory, spiral valves left exposed to the air all day yielding an abundance of *C. laciniatum* which were still alive and seemingly about as active as if taken from a shark just killed, and these worms if put into sea water may live for as long a period as forty-eight hours. In no case of an untreated shark which, on being injured by rough handling in my cars or by the collectors when first captured, was killed before it died from the effects of this handling *did I find the approach of death in the host killing the parasites*. We may therefore conclude that when, in a shark which has died very recently, or in one which has been killed because death seems approaching, there are found dead Cestodes, the worms have been killed by the drugs and not by the actual, or approaching, death of the host. Such cases have for this reason a sufficient value to be considered in the series. The objections against them are, first, that they do not represent individuals taken at random, and second, that it is of no value to show that the worms are killed in the sharks which do not sur-

vive the treatment while other worms are not killed in the sharks which do survive. It should be remembered, however, that such non-survivors do not represent the weakest individuals in any lot, for the strongest were probably the ones which fought hardest when put in the holder and such very active sharks received rougher handling and perhaps they sometimes died from this cause. With these reservations, the specimens found soon after death and those killed when about to die, may be cited as showing the immediate effect of the drug upon the parasites.

An important point, noted in some of the tables, is that about twenty-four hours after the dose of oil, that is, when the calomel was given, numbers of dead *Crossobothria* were squeezed from the anus as a shark was placed in the holder. Many entire worms of all sizes were thus obtained and these when placed in sea water slowly disintegrated, showing no signs of life, as they might have done if only stupefied by the oil.

Of the five sharks here recorded, No. 1 was not in good condition, though death did not seem near at hand, when it was killed the day after the oil was administered. It contained only dead *C. laciniatum*. Three days after the calomel was given the four remaining specimens were killed and examined, with the results which are shown in the table. They were all in good condition and the mucous membrane seemed quite normal. I may here state that my examinations of the spiral valves throughout this work have been most careful. In each case the outer wall was split longitudinally along one side and the cut continued down across the spiral folds to the opposite side. A similar cut was made across each fold along the middle of each half of the valve and the inner surface thus exposed as four parallel rows of triangular flaps, which were then examined one at a time under a lens. Where there was any such amount of chyme as to obscure the surface of the mucous membrane the valve was washed until clean and the washings examined in shallow dishes against a dark background.

Table 2 shows the results in five sharks, which were given a heavier dose of the oil of male fern, and the calomel after an interval of forty-eight hours. After the dose of oil, shark No. 1

TABLE 2

*5 sharks, captured before July 1, kept without food 3 to 4 weeks*

July 18, all sharks given 4 cc. each of oil of male fern  
 July 20, the three surviving sharks given 1 cc. each of calomel

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
July 19.....	No. 1	Dying	1 strobilla dead	Traces of oil in stomach
July 20.....	No. 2	Dead	No signs of cestodes	Digestive tract considerably decomposed
July 21.....	Nos. 3, 4 and 5	Killed	No signs of cestodes	Mucous mem- brane in good condition

TABLE 2a

*1 shark, captured about June 15, and kept without food*

July 1, given several cc. of oil of male fern diluted with ether.

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
July 5.....		Killed	No signs of cestodes	

seemed to be dying and was killed with the results indicated. No. 2 was found dead, but was a good deal decomposed and so the absence of worms may not mean much. The three surviving specimens, which were killed twenty-four hours after the calomel, were without any cestodes.

The results of these two experiments may be criticised on the ground that the sharks were not left alive long enough to show that more would not have died from the treatment, but my experience has shown that when the animals survived this treatment for two or three days the subsequent mortality was not likely to be greater than among any other sharks kept in confinement. Some of the sharks noted in other tables were kept alive a much longer time.

Before I began using the gelatin capsules a single shark which had been in captivity a few days was given some oil of male fern diluted with ether. How much actually got into the stomach I do not know, as some was spilled in pouring the mixture down the tube which was thrust into the oesophagus. No calomel was given. Five days later when the specimen was examined there were small traces of the oil still in the intestine and no worms were found.

TABLE 3

*20 sharks, captured July 4 to 7, kept without food*

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
July 25, all sharks given 2 cc. each of oil of male fern				
July 26.....	No. 1	Dead	9 scolices and bits of strobilæ, all dead	Decomposition not noticeable in intestine
July 26.....	No. 2	Dying	2 dead	Size of worms not recorded
July 26.....	No. 3	Dying	20 dead and in the faeces	Size of worms not recorded
July 26.....	No. 4	Dead	4 dead and in faeces	Decomposition of intestine not noticeable

July 26, the sixteen surviving sharks given 1 cc. each of calomel

July 27.....	No. 5	Dead	No worms	Decomposition just begun
July 28.....	No. 6	Dead	No worms	
July 28.....	No. 7	Dying	No worms	Mucous mem- brane normal
July 31 .....	No. 8	Killed	No worms	Mucous mem- brane normal

August 2, the twelve surviving sharks each fed on shark's flesh

August 7 .....	No. 9	Dead	No data	Considerably decomposed
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TABLE 3—(Continued)

August 15, the eleven surviving sharks each fed on shark's flesh

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
August 28. ....	No. 10	Killed	No worms	
August 28. ....	No. 11	Killed	No worms	
August 28. ....	No. 12	Killed	1 <i>Crossobothrium laciniatum</i>	
August 28. ....	No. 13	Killed	1 <i>Crossobothrium laciniatum</i>	
August 28. ....	No. 14	Killed	2 <i>Crossobothrium laciniatum</i> young	
			2 <i>Crossobothrium laciniatum</i> scolices	
			1 <i>Crossobothrium angustum</i>	

The six remaining sharks survived the treatment to this date and later, and were used for infection experiments. When examined they gave results as follows:

August 11. ....	No. 15	Dead	No worms	Badly decomposed
August 22. ....	No. 16	Dead	No data	Badly decomposed
August 29. ....	No. 17	Killed	No worms	Normal
August 31. ....	No. 18	Killed	1 <i>Crossobothrium laciniatum</i> medium size	
August 31. ....	No. 19	Killed	8 <i>C. laciniatum</i> large, scolices	
			5 <i>C. laciniatum</i> , small	
August 31. ....	No. 20	Killed	7 <i>C. laciniatum</i> , medium size	
			10 <i>C. laciniatum</i> , small	

Table 3 shows less satisfactory results than the foregoing, the net results being as follows: without parasites 11, found dead and so decomposed that no data were obtained 3; still infected 6.

If the survivors alone are considered the results are not nearly so good, for out of ten surviving specimens (Nos. 8, 10, 11, 12, 13, 14, 17, 18, 19, and 20) we have only four (Nos. 8, 10, 11, and 17) which are entirely free of cestodes, while there are six (Nos. 12, 13, 14, 18, 19, and 20) which are still infected. Of these six, Nos. 12, 13 and 18 have but a single parasite, so the chances are that some parasites have been eliminated, but Nos. 14, 19 and 20

show so many that one could not fairly claim any reduction as a result of the treatment. When, however, those specimens which were killed when they seemed in bad condition are taken into account, it is evident that many worms must have been eliminated, and the results indicate the elimination of many of the parasites from this lot of sharks, probably of all of them in those sharks which died from the treatment, but they also indicate that the dose as here given cannot be relied upon.

TABLE 4

*12 sharks, captured before July 2, kept without food*

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
July 28, each shark given 2 cc. oil of male fern				
July 29, each shark given $\frac{1}{2}$ cc. calomel				
August 30. ....	No. 1	Dying	No worms	Traces of oil and calomel in gut
August 1. ....	No. 2	Killed	No worms	Gut normal
August 3. ....	No. 3	Killed	No worms	Gut normal
August 4. ....	No. 4	Killed	No worms	Gut normal
August 16. ....	No. 5	Dead	No data	Decomposed
August 11. ....	No. 6	Killed	1 <i>C. laciniatum</i> , scolex	
August 11. ....	No. 7	Killed	3 <i>C. angustum</i> , small 3 <i>C. laciniatum</i> , small	

August 18, the five surviving sharks each fed on shark's flesh

August 28. ....	No. 8	Dead	No data	Decomposed
August 29. ....	No. 9	Killed	1 <i>C. laciniatum</i> , small	
August 30. ....	No. 10	Killed	2 <i>C. laciniatum</i> small found at very anterior end of spiral valve	
August 30. ....	No. 11	Killed	No worms	
August 30. ....	No. 12	Killed	2 <i>C. laciniatum</i> , large and with ripe proglottids 1 <i>C. laciniatum</i> , small	

Table 4 shows five cases of entirely successful expurgation (Nos. 1, 2, 3, 4 and 11). Specimens Nos. 9 and 10 had respectively one and two worms, while No. 12, since it has two large worms with ripe proglottids, does not justify the conclusion that the number of the parasites was even reduced, and No. 7 must be regarded in the same way.

TABLE 5

*12 sharks, captured July 25 to August 6, kept without food*

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
August 17, all sharks given 2 cc. each of oil of male fern				
August 18.....	No. 1	Dying	No worms	Dead worms from anus in handling
August 18.....	No. 2	Dying	No worms	
August 18.....	No. 3	Dead	No worms	Dead worms from anus in handling

August 18, the nine survivors given  $\frac{1}{2}$  cc. each of calomel

August 30.....	No. 4	Dying	No worms	Gut normal
August 31.....	No. 5	Killed	No worms	Gut normal
August 31.....	No. 6	Killed	No worms	Gut normal
August 31.....	No. 7	Killed	No worms	Gut normal
August 31.....	No. 8	Killed	No worms	Gut normal
August 31.....	No. 9	Killed	No worms	Gut normal
August 31.....	No. 10	Active	No data	Escaped
August 31.....	No. 11	Killed	8 <i>C. laciniatum</i> ,	medium size
August 31.....	No. 12	Killed	4 <i>C. laciniatum</i> ,	medium size

Table 5 has twelve sharks, less one which escaped during the handling. Of these eleven individuals, Nos. 5, 6, 7, 8 and 9 were without any infection, Nos. 1, 2, 3 and 4 did not survive, but showed that the drug had killed the parasites. Nos. 11 and 12 have respectively eight and four specimens of *C. laciniatum* and hence must be counted as against the effectiveness of the treatment.

It so happened that no shark, in which the *Crossobothria* survived, was killed among the first in any lot; as may be seen by reference to the dates on tables 1, 2, 3, 4 and 5. Thus by the 11th of August my records showed thirteen sharks which were killed when in perfectly good condition, six others which were killed, when it seemed that they were likely to die, and seven others, which died from the treatment and in not one of these had I found a single living *Crossobothrium*. This seemed to demonstrate the effectiveness of the single treatment with the oil of male fern, which was therefore continued, the sharks which survived it being kept for infection with the *S. polymorphus*. Later, when sharks began to appear in which some of the worms had survived, the season was so far advanced that there was neither

TABLE 6

*9 sharks, captured August 6 to 9, kept without food*

DATE	NO. GIVEN SHARK IN THIS SERIES	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE	REMARKS
August 18, all sharks given 2 cc. each of oil of male fern				
August 19, all sharks given $\frac{1}{2}$ cc. of calomel				
August 19, dead <i>Crossobothria</i> from anus of one specimen in handling				
August 30.....	No. 1	Dead	No worms	Decomposed a little
August 30.....	No. 2	Alive	No data	Escaped
August 30, gave the seven survivors 3 cc. each of oil of male fern				
September 1.....	No. 3	Dead	No worms	No noticeable decomposition
September 1.....	No. 4	Dead	No worms	No noticeable decomposition
September 2.....	No. 5	Killed	No worms	Gut normal
September 2.....	No. 6	Killed	No worms	Gut normal
September 2.....	No. 7	Killed	No worms	Gut normal
September 2.....	No. 8	Killed	No worms	Gut normal
September 2.....	No. 9	Killed	No worms	Gut normal

TABLE 7

*Showing the results of infection experiments*

DATE OF SHARK'S CAPTURE	NO. GIVEN IN THIS SERIES	DATE OF TREATMENT WITH O.M.F.	FED	INFECTED	FED	REMARKS
July 6....	No. 1	July 25		August 9	August 10	
July 6....	No. 2	August 1-3	August 4	August 8	August 10	
July 6....	No. 3	August 1-3	August 4	August 8	August 10	
July 1....	No. 4	August 1-3	August 10	August 11		
July 15....	No. 5	August 1-3	August 10	August 10		Fed at time of infection
July 6....	No. 6	August 1-3		August 9	August 10	
July 6....	No. 7	August 1-3		August 9	August 10	
July 1....	No. 8	August 1-3	August 10	August 11		
July 1....	No. 9	August 1-3	August 10	August 11		

TABLE 7—Continued

*Showing the results as in tables 1 to 6*

DATE	NO. GIVEN SHARK IN THIS SERIES AS ABOVE	CONDITION OF SHARK AT DEATH	CESTODES IN SPIRAL VALVE.	REMARKS
August 12....	No. 1	Dead	Many <i>S. polymorphus</i> found attached to the wall of the stomach and to the bit of the squeteague's stomach in which they were placed when introduced. The gut was slightly decomposed, but the larvae were still alive.	
August 16....	No. 2	Dead	No data. Badly decomposed	
August 31....	No. 3	Killed	Many small <i>Phoreiobothrium triloculatum</i> . See text p. 846	
September 1..	No. 4	Killed	3 <i>C. angustum</i> , small 3 <i>C. laciniatum</i> , small 7 <i>Phoreiobothrium triloculatum</i> .	
September 1..	No. 5	Killed	3 <i>C. laciniatum</i> , small.	
September 1..	No. 6	Killed	8 <i>C. laciniatum</i> , large with the long neck region 5 <i>C. laciniatum</i> , small	
September 1..	No. 7	Killed	10 <i>C. laciniatum</i> , small. 7 <i>C. laciniatum</i> , medium	
September 2..	No. 8	Killed	3 <i>C. angustum</i> , small Many <i>Phoreiobothrium triloculatum</i>	
September 2..	No. 9	Killed	1 <i>C. laciniatum</i> , scolex only. Many <i>Phoreiobothrium triloculatum</i>	

the time nor the material for trying a modification of the treatment upon any considerable number of sharks. Only nine sharks were differently experimented upon and the results obtained are represented in table 6, the net results of which are; without infection, 5; found dead but in sufficiently good condition to give reliable evidence that the worms had been killed by the drug, 3; escaped, 1.

The results of all the expurgation experiments may be summarized in the table which follows:

TABLE 8

*Showing net results from all experiments in treatment with oil of male fern*

TABLE REFERRED TO	SURVIVING SHARKS		SHARKS WHICH DIED UNDER THE TREATMENT, BUT IN WHICH THE WORMS WERE ABSENT OR HAD BEEN KILLED BY THE OIL OF MALE FERN		
	No infection	Still infected	Killed when seeming likely to die	Found dead	Total
1	4		1		5
2	3		1	1	5
3	4	6	3	4	17
4	5	4	1		10
5	5	2	4		11
6	5		3		8
	26	12	13	5	56

In this table, a few specimens which were found dead and so decomposed as not to yield reliable data are omitted. The specimens which survived treatment are of course most important and stand in the proportion of 26 for and 12 against the success of the attempted expurgation. Of the non-surviving sharks we have 13 specimens taken while still alive and all showing that worms were killed, as a result of the treatment and some time prior to the death of the shark. When one compares the number of worms found in these treated sharks with the tables and records showing their prevalence in sharks which have had no such treatment, it will be quite evident that a large proportion of the parasites must have been eliminated even by the single dose.

In those sharks which were given the double dose of o. m. f. (table 6) not a single cestode was discovered, but as only eight specimens were thus treated the number of trials is insufficient to show that even this treatment may be regarded as always effective. It is perhaps too much to expect a treatment that will be entirely effective in every instance, nor is such a treatment necessary, for a stray worm or two in one shark out of a dozen would still give us a result good enough for practical working purposes. Unsatisfactory though my results are, they do, I think, justify the belief that a little more experimenting along the line of repeating the dose one or more times would develop a method of treatment sufficiently effective for working purposes, i.e., will eliminate all the parasites, except in rare instances, without killing too many of the sharks. If such a method can be found, the sharks thus expurgated could be used in a variety of experiments. For example, by introducing young *Crossobothria* into a shark one might expect to find out more than we now know about the rate of growth and the maturing of proglottids in the cestoda, and another point worth examining would be the truth of the current idea that it is the inability of the cestode to survive in the wrong host which limits the habitat of a given species of parasite to a single host, or to a few closely related hosts. This latter point might, indeed, be investigated by the infection of sand sharks with larval forms other than the ones known to occur in that host, but where the normal infection is so great the examination of the specimens would be much easier if the host was first freed of all infection.

An insufficient supply of squeteague during the latter part of the second season made it impossible for me to make more than a few infections after the method of treatment, as above described, had been established. Only nine sharks were so infected and none of these was killed until about three weeks after the infection had been introduced. Hence the material is wanting to show my transitional stages between the *S. polymorphus* and the young specimens of *Phoreiobothrium* (figs. 9, 10 and 11) which I believe to have come from this larva. This lack of early stages resulted not because I chose, having only a limited number of

infections, to let them all run as long as possible, but because the sharks first infected were kept to run longest; while later infections were planned for the earlier stages, an arrangement which would give the most extensive time results in a limited time. When, however, the time came for infecting the sharks from which to secure the earlier stages, the squeteague could not be obtained in numbers sufficient to yield the larvae for infection purposes.

Of the nine specimens, two died in the cars and from one of these two no data were obtained, so there are only seven specimens from which entirely reliable conclusions can be drawn. The sharks used were all starved for three or four weeks after their capture and then given 2 cc. each of the oil, followed twenty-four hours later by about 0.5cc. of calomel. They were all specimens which had survived this treatment and the history of each individual previous to the time of the infection is given in the first half of table 7. Since there is no evidence connecting the genus *Crossobothrium* with the *S. polymorphus*, this table may also be used to furnish data on the success of the attempt at expurgation. The first part of the table gives the dates of capture and of the treatment with the drugs, between which events the sharks were kept in the cars without food. The dates at which they were fed are also shown for comparison with the dates of infection. Each shark is numbered, as in the previous tables, and these numbers are repeated in the second half of this table where the results of the examinations for parasites are tabulated in a manner similar to that followed in the tables for treatment with the oil.

In introducing the *S. polymorphus* into these sharks I took the portion of the cystic duct containing the larvae from twelve squeteague, and placed these in the little sac obtained by cutting off the end of a squeteague's stomach. This sac was turned wrong side out to avoid any possible injury from direct contact with the mucous membrane of the squeteague. The infection thus prepared was introduced into the shark's stomach by pushing it through a piece of iron pipe having a diameter sufficient to admit such an object without undue pressure. For food, I used

the flesh of another sand shark in preference to that of a teleost, since there is the minimum likelihood of finding any cestode larvae in the flesh of a large shark; whereas a teleost may, in addition to its normal parasites, contain almost any thing in the way of a 'xenositic' larva. All the sharks were fed shortly before or after their infection, as is shown in the first half of the table.

The latter part of table 7 shows the results when these same sharks were examined for parasites, and by reference to the number given each specimen, one may follow any one fish through the two parts of the table. The only shark examined soon after the infection is No. 1, which was found dead. In this shark, specimens of the *S. polymorphus* were found adhering to the surface of the shark's stomach and to the remains of the bit of squeteague's stomach in which they had been introduced. Although this specimen was not found until decomposition was quite in evidence, these larvae still showed some slight movements and had therefore survived in the stomach for a period of three days. Shark No. 2 died during my absence from Woods Hole and was so badly decomposed when found that no data were obtained. In the spiral valve of specimen No. 3 there were a very large number of young *Phoreiobothrium triloculatum* (figs. 9, 10 and 11). I collected and preserved some thirty-five of these worms, but this number represents only a small part of those present. Owing to their minuteness when only the delicate posterior end can be seen protruding from between the villi of the intestine and the tenacity with which their powerful hooks enable them to retain their hold, these larvae are often very difficult to detach from the walls of the spiral valve, though they may be large enough to be easily recognized. There must have been present in the shark many more than I collected; for taking into account the ones actually seen but not collected, I estimated at the time that there were a good many more than one hundred of these young worms in this single fish. A fact of perhaps more importance than their numbers is that in any one shark they were all in the same stage of development.

Shark No. 4 shows three specimens of *C. laciniatum* which are to be regarded as worms which survived the expurgation treat-

ment. Seven small specimens of *P. triloculatum* were collected, but in this case it was evident that the valve contained a much smaller number of these than the previous one. My notes taken at the time state that no more were seen in addition to those actually collected, although there may have been some toward the anterior end where the villi are longest. Specimens Nos. 5, 6 and 7 all present evidence against the effectiveness of the vermifuge. In 6 and 7 the numbers of surviving cestodes is so great that no certain effect from the drug is indicated. There is perhaps some significance in the fact that here, as in some other cases where the worms are found surviving the effects of the oil, the proportion of young to adult specimens is somewhat increased. This may indicate that the drug is more effective with the large worms which have long bodies extending among the folds of the valve than with very young ones which may often be almost buried among the villi and so escape the full effects of the drug. In this table there are recorded from specimens 4, 5, 6, 7, 8 and 9 a total of 43 surviving *Crossobothria*. Of these only 9 are beyond the period of segmentation into proglottids and from what we know of the proportions in which the young and old are commonly found it would seem that the drug has destroyed more adult than young worms. This conclusion is of course based upon my belief that these young *Crossobothria* have not come from the introduced *S. polymorphus*.

In sharks Nos. 8 and 9 the same condition was found as in No. 3, namely, so many specimens of small *P. triloculatum* that the counting them was an impossible task. Later when I examined very carefully the preserved specimens I found that the *Phoreiobothria* from any one shark were of uniform size, but that when those from different sharks were compared there was some difference in the size attained. For instance those from specimen 9 are almost twice the size of the ones from specimen 3. This difference in size is noticeable only in the body portion of the larvae, the scolices being of very uniform size.

The presence of the *C. laciniatum* and a few *C. angustum*, although there are more young specimens among them than one would expect to find in sharks taken at random, does not seem

to indicate anything except the ineffectiveness of the attempt at expurgation. There can be no interpretation of the facts which would show that the *S. polymorphus* had given rise to these *Crossobothria*.

Despite the lack of intervening stages between the *S. polymorphus* and the specimens of *P. triloculatum*, which I found in these sharks, I think there is some pretty good evidence that the latter have developed from the introduced *S. polymorphus*. First, the examination by myself, and also by Linton, of a large number of sand sharks at various times has never shown that young or adult specimens of *Phoreiobothrium triloculatum* are to be found as regular parasites of this shark, or as frequent 'xenosites.' I have never found this form in any sharks except those which I infected with the *S. polymorphus*. It is very probable, however, that one might at any time find stray specimens of this worm, since the squeteague is a not uncommon food of this shark. The failure to find it in any of the sharks I have examined would indicate that it does not often survive, when introduced in nature along with the squeteague, and in my experiment only a small proportion of the larvae introduced have survived. Second, the specimens of *P. triloculatum* which I found in any one shark were of very uniform size, and unless we suppose that there is some limit to their growth, when in an abnormal environment (the wrong host), this would indicate that they all entered the shark at about the same time. In the third place this conclusion is also in line with the results of Monticelli ('88) who has shown that the *S. polymorphus* of European waters develops into the genus *Calliobothrium*, which belongs to the same family as *Phoreiobothrium*, a fact which is further discussed in the portion of this paper which deals with the nature of the *S. polymorphus*.

Attention should here be directed to one point which has perhaps suggested itself from the examination of the figs. 7-13. This is that the specimens of the young *P. triloculatum* (figs. 9 and 10) are considerably smaller than some of the specimens of the *S. polymorphus*, from which I suppose them to have developed. This appeared to me at first a most serious objection, though upon further consideration it does not seem an insur-

mountable one. The ragged and irregularly constricted ends of some of the specimens (figs. 7 and 8) suggest that part of the body is being lost, as in the case in the development of the strobila in those forms which have a typical bladder-worm stage. Again, while the body region is smaller than that of the larger specimens of the *S. polymorphus* (fig. 12) the scolex of the young *P. triloculatum* (figs. 9, 10 and 11) is considerably larger than that of the *S. polymorphus*. I should also add that fig. 12 represents one of the very largest of the larvae and has been killed under pressure to flatten the body and make it more suitable for a whole mount whereas the specimens of *P. triloculatum* were killed without pressure and the body has remained cylindrical. The *S. polymorphus* reaches this size (fig. 12) only in the cystic duct and the smaller specimens present a lesser disparity in size. I am inclined to think that in the case of such large specimens portions of the *S. polymorphus* body may be moulted off just as in the case of the bladder portion in the cysticercus. One constant feature of the larger specimens of the *S. polymorphus* has, perhaps, some significance in this connection. It is the occurrence of a denser region which terminates abruptly a little way behind the scolex (fig. 12). In a specimen stained and mounted whole after the carbonate of lime granules, which occupy so much of the parenchyma in the body region, have been dissolved out, one can distinguish this region as having the same denser appearance as the tissue in the body of the young *Phoreiobothria* (figs. 10 and 12) or the region of proglottid formation in young specimens of *C. laciniatum*. It is possible that the scolex and this region of the *S. polymorphus* are the most important in the formation of the adult worm and that part or the whole of the body region may be lost. It seems probable that larvae like the *S. polymorphus* have been derived from larvae of the cysticercus type and, if this be the case, it would not be surprising to have a part of the body region lost at this point in the development.

## SUMMARY

Experimental infections of the sand shark (*Carcharias littoralis*) with the cestode larva known as the *Scolex polymorphus* indicate that the larvae used for these experiments developed into the species *Phoreiobothrium triloculatum*. It seems clear that the common tapeworm (*Crossobothrium laciniatum*) of this shark cannot come from the *Scolex polymorphus*, even though this larval type may represent the young of a number of cestodes, a possibility which is referred to in the third section of this paper.

Starving the sharks had no effect upon the cestodes, but by means of treatment with the oil of male fern followed by calomel the great majority of the parasites were eliminated before the sharks were artificially infected. It seems probable that by a little more experimentation a method of treatment could be secured, which, for eliminating these parasites, would be sufficiently effective for all working purposes.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Outline of a living specimen of the *Scolex polymorphus*. The stippled areas just behind the four bothria show the location of the faint red pigment spots seen in some specimens. Magnified about 45 diameters.

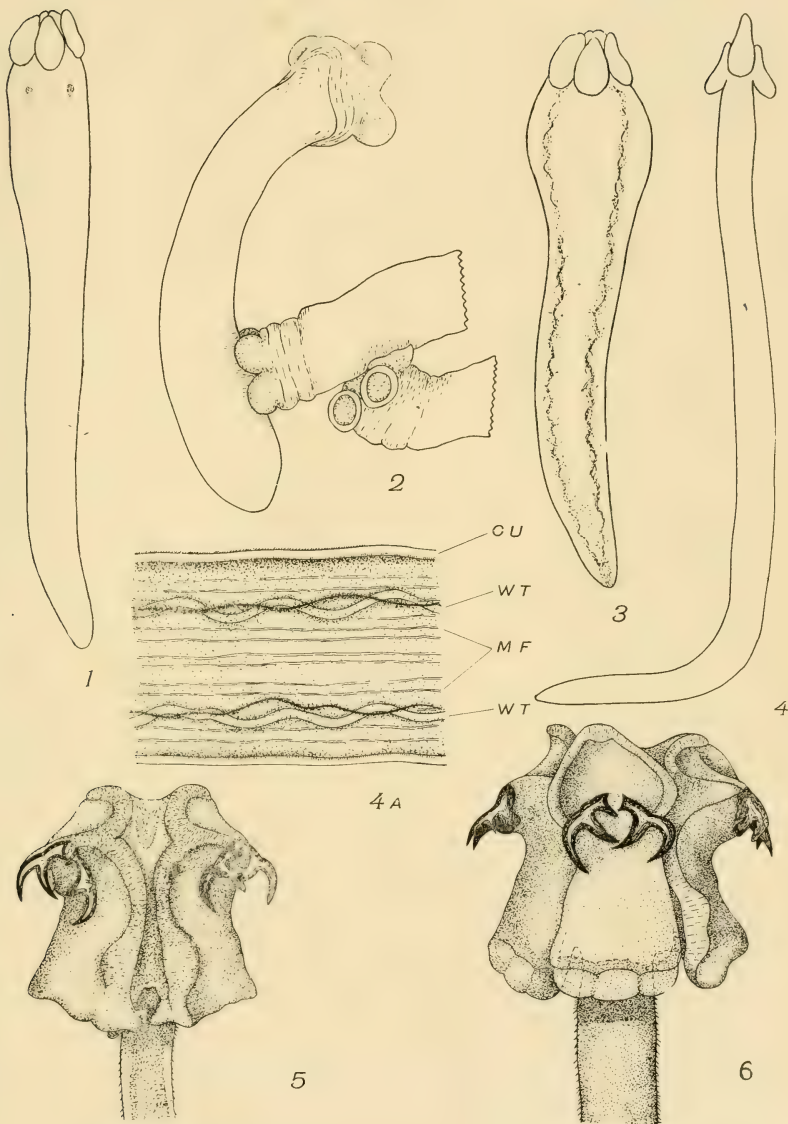
2 Specimens of the *Scolex polymorphus* drawn from the living specimen to show the characteristic mode of attaching with all four suckers to the bottom of a dish in which they are being examined and the manner in which they fasten to one another. Magnified about 45 diameters.

3 and 4 Outlines of the *Scolex polymorphus* drawn from living specimens to show other characteristic shapes. The area occupied by the large and small vascular trunks of either side and the terminal vesicle are added to fig. 3 as they appear in a stained specimen. The filiform appearance shown in fig. 4 is often seen as the larvae draw themselves over a surface by the characteristic movements of their bothria. Magnified about 45 diameters.

4a From a specimen stained and mounted whole, showing a portion of the body region on a large scale. The cuticle (*cu*), the larger and smaller excretory trunks (*wt*) and the conspicuous longitudinal muscle fibres (*mf*) are shown. The stippling indicates the distribution of nuclei in the parenchyma as it appears in optical section. Along part of the upper margin are shown the minute projections which occur upon the cuticle in the posterior part of the scolex region and for a short distance along the body, finally giving place to the smooth cuticle as shown in the figure. Magnified about 200 diameters.

5 Scolex of a young *Phoreiobothrium triloculatum*, taken from a sand shark artificially infected with *Scolex polymorphus* (see shark no. 3 of table 7). This view shows the division between two neighboring bothria along the mid-line of the figure. The characteristic pairs of hooks are shown attached along the line separating the upper and middle loculi of each bothrium. In this figure the subdivision of the posterior margin of the bothrium into three loculi, from which the specific name is derived, is not seen because the contraction of the lower margin brings their surface at right angles to the general surface of the bothrium. The area on the mid-line of the figure toward the anterior end of the scolex, and marked by the closer stippling, appears in some specimens and may represent the 'myzorhynchus' of the *Scolex polymorphus*. The minute spikes protruding from the neck region of the cuticle are shown in profile only. Magnified about 90 diameters.

6 The same as the last figure. One of the bothria is shown in front view and the three posterior loculi are expanded so as to show clearly. Magnified about 100 diameters.



## PLATE 2

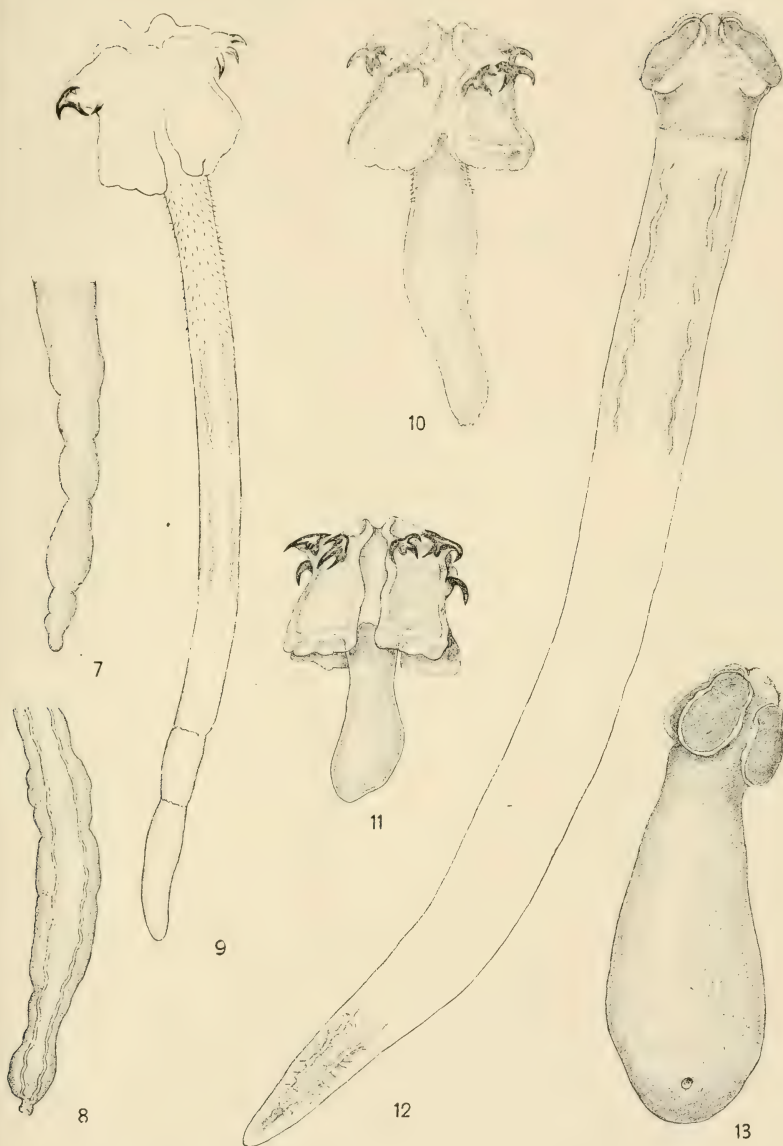
### EXPLANATION OF FIGURES

7 and 8 The posterior end of a young specimen of *Phoreiobothrium* from a shark artificially infected with the *Scolex polymorphus*. These figures show the formation, either of psuedo-proglottids which are molted, or an irregular beginning of true proglottid formation like that of *Crossobothrium laciniatum* (Curtis, '06). The figure shows so much irregularity that the process does not seem like true proglottid formation and the posterior end shows a peculiar outline which probably indicates that part of the specimen has been lost. Only the larger pair of the two water trunks is shown. Magnified about 70 diameters.

9 One of the largest specimens of *Phoreiobothrium triloculatum* which was secured from the artificially infected sharks (see shark no. 8 of table 7). This is one of the few specimens which showed a segmentation of the posterior end sufficiently regular for comparison with the formation of the posterior proglottids in *Crossobothrium laciniatum*. The scolex of the specimen is shown in outline only. The area of the minute spikes is shown and back of this the two pairs of water trunks which in such a view are superposed throughout the length of the body, are here shown in successive regions. Magnified about 70 diameters.

10 and 11 Two of the smallest of the specimens of *Phoreiobothrium triloculatum* obtained from the artificially infected sharks (see shark no. 3 of table 7). The specimens represented in fig. 10 was ragged at the posterior end as though portions had been detached. Magnified about 70 diameters.

12 and 13 Show in more detail the features of the *Scolex polymorphus*. In fig. 12 the 'myzorhynchus' or proboscis-like structure is shown at the anterior end between the four bothria. The larger and smaller water vascular trunks are shown in successive regions and their convergence toward the posterior end where the vesels become irregular and branched so that they can only be followed as an area of differentiation in the parenchyma. Each bothrium consists of a denser portion divided into three regions or loculi in the older specimens (fig. 12), and in those slightly smaller often showing only the line of division at the anterior end (fig. 13). In fig. 13 the opening of the terminal vesicle of the water tubes is seen in surface view, the posterior end of the specimen being slightly turned toward the observer. Magnified about 70 diameters.





# THE LIMITS OF HEREDITARY CONTROL IN ARMADILLO QUADRUPLTS: A STUDY OF BLASTOGENIC VARIATION

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FIVE TEXT FIGURES AND EIGHT PLATES

## CONTENTS

Introduction.....	855
Morphology of the integument.....	861
Meristic variation of the elements of the nine bands of armor.....	865
A Variation in the banded region as a whole.....	865
B Comparative variability of males and females.....	868
C Variation in the individual bands.....	870
D Fraternal correlation in the banded region as a whole; an index of the limits of hereditary control.....	873
E Fraternal correlation in the individual bands.....	880
Atypical variation in the individual elements and in the bands.....	883
A Scute 'abnormalities' in the species; their distribution and frequency.....	883
B Hereditary control in connection with scute 'abnormalities'.....	888
C Band 'abnormalities' in the species; their distribution and frequency.....	893
D Hereditary control in connection with band 'abnormalities'.....	897
Pairing, an intra-fraternal correlation; and its bearing on the problem of hereditary control.....	905
The hereditary control of sex.....	910
General considerations.....	911
Summary.....	914
Bibliography.....	917

## INTRODUCTION

*To what extent or within what limits are the definitive characters of the individual determined at the time of fertilization and in how far are the minutiae of organic structure to be considered as the product of individual variability beyond the limits of hereditary control? No more fundamental question could well be raised, and none more difficult of solution. The question has recurred*

in various guises, but the underlying inquiry has remained unchanged. In one of its older forms the question is phrased: "What is the relative potency of nature and nurture in development?" In some of its more modern phases it appears in terms of 'predetermination versus epigenesis,' 'blastogenic versus somatogenic variation,' and 'heredity versus environment.'

In spite of the antiquity of the problem very little direct evidence has appeared for its solution. So far as we have been able to ascertain, the only facts that seem to throw any clear light on the situation are those furnished by cases of human 'identical' twins. As long ago as 1875 Galton showed his appreciation of the value of such data in his paper entitled "The history of twins, as a criterion of the relative powers of nature and nurture." In a subsequent paper (Galton, '92) he made use of finger-prints as criteria for distinguishing between twins and as a suitable character for testing the degree of likeness and unlikeness of such pairs. That he had a deep insight into the underlying fundamental problems involved in the situation is shown by his remark: "It may be mentioned that I have an inquiry in view which has not yet been fairly begun, namely to determine the minutest biological unit that may be hereditarily transmissible. The minutiae in the finger-prints of twins seem suitable objects for this purpose."

In 1904, Wilder, interested in the same problem, presented a comprehensive review of the whole subject in his paper on "Duplicate twins and double monsters." His results are of great interest and will, we hope, assume a still greater significance in the light of the facts here presented. Wilder discovered a surprising degree of resemblance between the palm and between the sole patterns of duplicate twins and was able, he thought, to classify twins as 'fraternal' or 'duplicate' on the basis of this resemblance. In addition, the following phenomena were observed in the majority of cases, but were not universal:

"(1) A bilateral correspondence in the palms and soles of each individual of a set.

"(2) A reversal of the finger patterns in either of the right or the left indices.

“(3) Differences occur more frequently on the left side.”

In his concluding paragraph, here quoted, are given in concise form the chief results and conclusions derived from his studies of duplicate twins:

The influence of the germ-plasm and its mechanism (i.e., the direct control exercised by heredity) is exerted upon the friction-skin surfaces only so far as concerns the general configuration, i.e., the main lines, the patterns and other similar features; the individual ridges and their details (minutiae) are apparently under the control of individual mechanical laws to which they are subjected during growth. *Have we then arrived at the limit of the control of the predetermining mechanism beyond which mechanical laws are alone operative; and is it then possible to hold that the modifications in this latter field are the results of individual experience, and that they are similar in the various members of a given species solely because of similar environment?* To these and similar questions we can give no answer at present; yet it seems likely that in the general subject of palm and sole markings, not only in man but in other mammals as well, we have a set of easily observed and very significant data which may yield important results to future investigators.

In addition to his data on palm and sole patterns Wilder furnishes us with rather elaborate physical measurements of four sets of duplicates. He realizes, however, that dimensional data are “far less determinative than are other characters employed, since they are liable to fluctuations through numerous causes, both internal and external, and it could hardly be expected that the similarities here would be very striking.” Yet some most striking physical resemblances have been brought out by different authors. Vernon ('03), for example, gives the data for two pairs of identical twins, one of which, aged twenty-three, showed an average per cent difference of 0.28 per cent; while the other, aged twelve, a somewhat greater difference of 0.71 per cent. Weismann presents the data on one pair of twin brothers, aged seventeen, who showed a per cent difference of 2.2, nearly ten times that of Vernon's first pair and about three times that of his second.

Wilder's measurements include a much wider range of characters than do the others cited and are therefore probably of somewhat greater value. Table 1 presents a brief summary of his data.

TABLE 1

*Showing physical statistics of Wilder's twins*

SET	NUMBER OF CHARACTERS	PER CENT DIFFERENCES	AGE IN YEARS
I.....	27	2.39	21.10
II.....	27	2.35	17.11
III.....	27	1.64	17.10
IV.....	50	1.76	17.11
Mean.....		2.03	18.1

Although Wilder realizes that dimensional and other physical measurements "should be taken during the younger life or at least before there is any marked difference in the experience," yet he apparently considers that in all of his four sets, whose ages range from seventeen to twenty-one years, "these conditions are met with, as they are all those of young people." Seventeen or more years of post-natal life would seem, however, to be sufficient for the operation of nutritional differences, some of which might tend to cause originally identical characters to diverge, and others, originally divergent characters to converge. It would seem to be inadvisable then to attempt to draw the line between nature and nurture on the basis of physical characters so subject to modification by environment, for a variation in nutrition alone might readily produce all of the differences shown in the sets of physical measurements cited. Nutritional differences doubtless manifest themselves even before birth, and hence would tend to vitiate the results of physical measurements taken on new-born duplicates even if such were available. Consequently it would seem advisable to limit investigation to those characters which reach a definitive condition at an early period and which are subject to little or no modifications due to nutrition. The patterns of the friction ridges of the palm and sole are characters of this sort and should give highly reliable data as to the strength of hereditary control. But even this data, interesting and suggestive as it is, can be accepted only with a considerable amount of reservation; for it has, in common with all other data derived from

a comparison of human identical twins, the fundamental weakness that it is based on an assumption which is clearly beyond the possibility of proof. Because certain twins exhibit a most striking resemblance, it is assumed that they have been derived from a single fertilized egg. As Weismann ('02) expresses it, "*Wir haben nun allen Grund, die erste Art von Zwillingen von zwei verschiedenen Eizellen abzuleiten, die letztere Art von einer einzigen, welche erst nach der Befruchtung durch eine Samenzelle sich in zwei Eier getheilt hat.*"

Were it possible in a number of cases to determine by examination of the placental relationships of new-born twins whether they were duplicates or fraternal, and were it also possible to obtain data on these cases whose uterine history was known, we would have facts from which we could with confidence draw conclusions. Unfortunately, however, although monochorial twins have been observed at birth, no interest has been manifested in their resemblances. In view of the lack of satisfactory criteria as to the origin of the twins investigated, the writers on these subjects have reasoned backwards from the facts of resemblance to the assumption of common origin, a procedure far from safe, but doubtless justified by circumstances. An arbitrary criterion is thus set up for the classification of twins; and those that come up to specifications are classed as 'duplicates,' while those that fail to meet the arbitrary requirements are relegated to the rank of 'fraternals.' One cannot but be impressed, as he reads Wilder's monograph, with the author's feelings of uncertainty as he attempts to classify certain pairs. The following extracts indicate his attitude:

No. VII. This case has caused me considerable trouble, owing to the preconceived notion that the marks ought to be found identical. The family emphasized the facial resemblance of these twins and when I first saw them they certainly looked much alike. One was, however, an inch taller than the other, and the facial resemblance, after a short acquaintance did not seem as great. . . . The case is plainly one of fraternal twins that resemble one another somewhat more than the average.

No. XIII. According to personal appearance these should be duplicates. I have never seen them, but the one who took the prints wrote: 'The Misses\*— are so similar in coloring, figure and features

that even their best friends confuse them.' It must be confessed, however, that the differences in the formulae cannot be reconciled, and that the palms are, and remain, in respect to the main lines, very different.

\* In the light of the results presented below we are inclined to believe that Wilder was not justified in thus arbitrarily excluding from the category of 'duplicates' such cases as those referred to, for we have found not a few sets of armadillo fetuses which exhibit greater differences than some of Wilder's so-called 'fraternals.' Hence, although the results of his studies are valuable and highly suggestive, they are insecurely founded and therefore cannot be applied to the solution of the problem of the limits of hereditary control.

The material which forms the basis of the present investigation consists of a collection of advanced sets of fetuses (removed from the uterus with all of their placental connections intact) of a species of mammal, *Tatu novemcinctum*, in which we have demonstrated conclusively the existence of specific polyembryony; and hence all sets of embryos, whether strikingly similar or not, are known to be the product of the division of a single fertilized egg. The basic assumption involved in the case of human duplicate twins is thus obviated, and at the same time it is possible to eliminate the factor of a diverse post-natal environmental experience by examining unborn fetuses, whose inter-relationships are shown by their placental connections. In the scutes of the banded region we have characters little if at all subject, even during gestation, to environmental control. We plan in the present paper to present an intensive study of the phenomena of blastogenic variation as exhibited by these integumentary elements, limiting our present investigations to the well defined banded region, believing that the conclusions arrived at from the study of one region will prove to be generally applicable, and that what is true for one character or set of characters will be found to apply in a general way to the whole organism.

In order that there may be no misunderstanding as to the kind of variates we are dealing with, it seems advisable to present in abbreviated form the results of a study of the morphology of the integument and of the variability of its elements as exhibited by

a large sample of the species. Without this data one would scarcely be in a position to appreciate the degree of resemblance or difference that exist among the foetuses of the different sets.

Owing to the existence of an extensive curio industry, making a specialty of baskets shaped from the shells of armadillos, there has been afforded an exceptional opportunity for gathering a large mass of data on the variability of the species. By availing ourselves of the large stocks of basket-shells in the hands of various dealers we have been able to examine 1768 individuals for scute and band 'abnormalities' and to count the scutes in the banded region of over 500 shells, including those of all males and females sent to us alive.

#### MORPHOLOGY OF THE INTEGUMENT

The integument is one of the most characteristic features of the anatomy of the armadillo. For the most part it consists of a series of bony plates which are arranged so closely together as to form an almost continuous armor, especially on the dorsal and lateral parts of the body. When attacked the animal is able to retract itself well within this shell-like structure, much after the manner of a turtle, and although the belly and legs do not possess an armor, in the strict sense of the word, yet even here the skin is studded with horny scutes and the feet are armed with powerful claws. Altogether the integument of the armadillo forms a protective structure of high efficiency in an otherwise defenseless animal.

In our species, *Tatu novemcinctum*, five of the so-called armor shields described for armadillos are present. These are the cephalic, covering the front of the head; the scapular, overlying the shoulders; the thoraco-lumbar or banded region (sometimes called the movable zones), consisting of nine bands or incomplete rings; the pelvic, covering the hips; and finally the caudal shield, which consists of a series of rings surrounding the tail (fig. 17).

The elements composing the armor exhibit in each of the shields a somewhat different and more or less characteristic arrangement; but since in this paper we are concerned with the study of varia-

tion and heredity in but one of these shields, it is not necessary to enter upon a description of the elements of the others, except in so far as such an account would be of help in understanding the character of the elements in the particular region in question.

The nine bands are united to one another by strips of flexible skin which permit considerable motion in this part of the armor. The first band is attached in a similar manner to the scapular shield, while the integument joining the ninth band to the pelvic shield may be present only toward the ends of the band, in which case the middle of the band is firmly united to the shield. The soft parts of the body lying directly beneath the strips of flexible skin are not exposed, because of the fact that the bands overlap one another for a distance equal to about one-third their width, that is, the posterior margin of any band overlaps the anterior third of the succeeding band. On account of this overlapping the banded region as a whole presents a distinctly testudinate appearance.

Each band is composed of a number of elements, of which there are three kinds: (1) the thick, bony, dermal plates covering the under surface of the band and constituting its main body; (2) the thin, horny, epidermal scutes which cover the posterior exposed part of the band; and (3) the associated hair group.

Each bony plate is oblong in outline, with its long axis constituting the width of the band, and in the adult animal has an average width and length of 6 mm. and 30 mm., respectively. As we shall see later, the number of these plates varies in different bands as does also the number for the same band in different individuals, but in round numbers there are on the average about 62 plates to the band.

The epidermal scutes, unlike the underlying bony plates, are of two types, which we may call primary and secondary. The first of these is represented by a wedge-shaped area having a slightly convex upper surface, and with its base forming a part of the posterior margin of the band (fig. 20). The base of each scute has two notches which are situated so as to divide the margin into three areas of about equal width. In reality the notches are but the mouths of hair pits located in the underlying bony plate

and from which fine hairs extend. The plate also has two other marginal hair pits containing hairs, one situated well toward each corner of the base; but these emerge from beneath the scute, and consequently the margin of the latter shows no corresponding emargination, as in the case of the more centrally located pits.

The secondary scutes are also more or less wedge-shaped, but, unlike the primary ones, have their bases directed toward the anterior margin of the band (fig. 20). In brief, their bases form the anterior limit of the exposed part of the band. The apex of the wedge is blunt and forms a small part of the posterior margin of the band. Through the median axis of the scute a faint groove extends from the middle of its base to a point near the apex. Upon the removal of the scute it is found that the groove is due to the depression of the suture between the two adjacent bony plates, directly above which the scute is situated. The secondary scutes are clearly composite structures; that is, they have been formed during the process of their evolution by the union of some three or four elemental units of regions such as are seen in the scapular and pelvic shields, which exhibit conditions more primitive than that of the bands.

In addition to the four hair-pits already alluded to, several others belonging to the associated hair group appear on the upper surface of the plate. The most prominent of these is a row of six or seven extending along each side of the primary scute marking. These pits possess very fine hairs which often extend above the surface of the band, especially in young animals. There are also faint indications of other hair-pits, both on the convex area of the plate as well as on its anterior unexposed third, but it is entirely beyond the scope of this paper to enter into a detailed description of them. It is sufficient here merely to note that each plate corresponds to a rather distinct hair area of other mammals, and consequently has a definite number of hairs included within its limits.

It will be evident from the foregoing account that for each primary scute there is a corresponding plate in which is imbedded a definite group of hairs. A count of the primary scutes will therefore also give a count of the plates and of the hair groups.

This whole complex will be, for purposes of brevity, designated the 'scute,' because the scute is the index of all of the elements entering into the complex.

It has been suggested above that the bands have evolved from a more primitive condition of the integument—one in which the bony elements were not necessarily arranged into definite rows. This becomes obvious when one studies such adjacent parts as the scapular and pelvic shields. In each of these regions the general arrangement is much the same, but the transitional condition is more clearly brought out in the pelvic shield, and we shall therefore confine our account to this part.

In the central part of the pelvic shield the bony plates are hexagonal in outline, and are so closely crowded together that a solid bony structure is formed (fig. 16). At best the plates can only be said to be imperfectly arranged into rows. Toward the anterior margin of the shield, however, the serial arrangement into rows becomes more evident, and all of the plates show a distinct tendency to elongate in the antero-posterior direction. In the extreme anterior margin of the shield, or the part corresponding to a tenth band, they become distinctly oblong and greatly resemble those of the true bands. In many of them, however, one can still detect their hexagonal shape, although the anterior and posterior ends show but faint indications of their double-sided nature. Even in the last of the true bands, the ninth, the posterior end of many of the plates is still in the form of an obtuse angle.

On the upper surface of this same region of the armor the scutes show corresponding transitional conditions. The primary scutes are here slightly elevated above the more numerous secondaries and have their posterior ends capped with small white or unpigmented areas, giving to the entire pelvic shield a distinctly pebbled effect (fig. 21). As one passes forward on the shield there is noted a gradual change in the primaries from small polygonal areas to those with characteristic wedge-shaped outlines. This is particularly noticeable on the lateral aspects of the shield.

In the typical regions of the pelvic shield the secondary scutes are more numerous than in the bands. In place of the single

secondary we have here some four or five elemental units. In the neighborhood of the bands these gradually fuse together, and in the region immediately adjacent to the ninth band are usually typified by two pieces, one, a regular trapezoid in shape, forming the anterior part, and the other, triangular in outline, abutting against this posteriorly. In the true bands these two pieces fuse to form the characteristic secondary scute.

A great deal of interesting data might be given concerning the much more primitive condition of the integumentary elements as seen on the ventral side of the animal, but it must be sufficient merely to suggest one or two of their more salient features. On the belly the horny structures only are present, and these are associated with a group of five or six hairs, or even more. On the legs some few of the larger horny elements, especially those which have a tendency to be arranged into definite rows, are underlaid with true bony plates. Evidently the hair group is the most primitive element of the complex, and in connection with these elements have grown up the scutes and plates.

#### MERISTIC VARIATION OF THE ELEMENTS OF THE NINE BANDS OF ARMOR

##### *A. Variation in the banded region as a whole*

It is our purpose to present in this section only so much of the results of our studies of the variability of the species as appears to be prerequisite for an understanding of the phenomenon of fraternal correlation. A concise tabulation of the distribution of the variates and a determination of the principal variation constants should serve the purpose in view as well as would a more detailed account.

That the characters dealt with show a high degree of variability is obvious if one examine the array exhibited in table 2. The number of scutes in the banded region vary all the way from 517 to 625, a range of nearly 20 per cent. In connection with our studies on fraternal correlation it will be of value to bear in mind this high species range of variability. In table 2 is presented the array of individuals investigated, comprising 508 adults and shells.

TABLE 2

*Showing distribution of frequency of scutes in the banded region of 508 adults*

NUMBER OF SCUTES	FREQUENCY	NUMBER OF SCUTES	FREQUENCY	NUMBER OF SCUTES	FREQUENCY
517	1	554	8	591	1
518	0	555	9	592	0
519	1	556	11	593	1
520	1	557	10	594	0
521	2	558	21	595	0
522	1	559	13	596	1
523	1	560	10	597	0
524	0	561	18	598	0
525	1	562	15	599	0
526	0	563	19	600	0
527	2	564	6	601	0
528	5	565	15	602	0
529	0	566	12	603	2
530	0	567	10	604	0
531	1	568	11	605	0
532	3	569	8	606	0
533	3	570	12	607	1
534	7	571	12	608	0
535	2	572	8	609	0
536	2	573	7	610	0
537	3	574	8	611	0
538	7	575	4	612	0
539	6	576	8	613	0
540	5	577	7	614	0
541	10	578	5	615	0
542	5	579	7	616	0
543	6	580	5	617	0
544	16	581	3	618	0
545	5	582	2	619	0
546	7	583	3	620	0
547	18	584	3	621	0
548	14	585	3	622	0
549	17	586	0	623	0
550	13	587	4	624	0
551	7	588	0	625	1
552	12	589	3		
553	15	590	2		

In grouping the variates shown in the table for purposes of seriation we have decided to fix the group size on as logical a basis as possible. The average range of variability of the sets of quadruplets is eight scutes. We shall therefore seriate the array in groups of eight for reasons which will become clear later. The polygon of variation obtained by this grouping represents a close

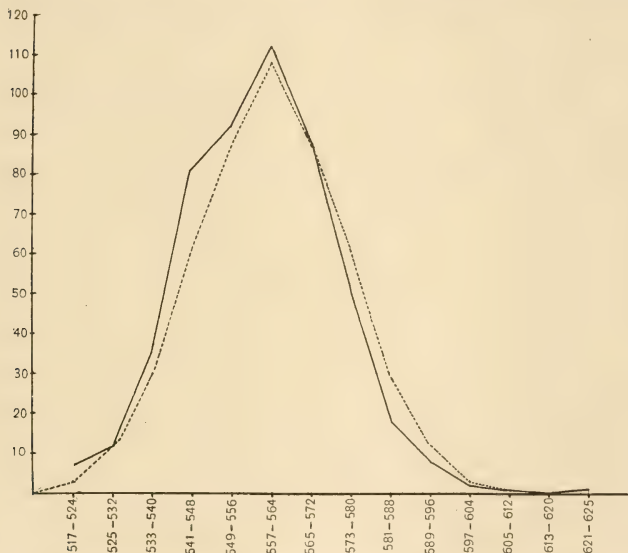


Fig. 1 Polygon of variation for the total number of scutes in the nine bands, as determined from a seriation of 508 individuals. Class range = 8 scutes. The solid line represents the observational and the broken line the theoretical normal curve. In this and the succeeding figure the abscissae refer to number of scutes and the ordinates to the number of individuals.

approximation to a normal array as may be seen from a comparison of the observed and the calculated curve (fig. 1).

The three important constants of the frequency polygon practically coincide; the median being 558; the mean, 558.2; and the mode, calculated from these two constants, 557.6. On the ex-

treme right of the polygon will be noted several extreme variates, so far separated from the others as to be examples of discontinuous variation. The rejection of these extreme variates would render the three constants, mean, mode and median, practically identical, but it would seem inadvisable to take any liberties with the data. Rather we would prefer to accept a close approximation to the normal curve of frequency as an indication that we are dealing with a case of chance variation, little if at all disturbed by complicating factors.

From the above array have been calculated the standard deviation,  $14.89 \pm 0.31$  scutes; and the coefficient of variability,  $2.685 \pm 0.32$  per cent.

In addition to these facts we are also led by analogy to infer that we have in the scutes of the nine bands of armor a variant which is inherited in the blended fashion. This inference is borne out by the evidence derived from an examination of the mothers of the various sets of quadruplets (table 6). Of the fathers we unfortunately know nothing.

### *B. Comparative variability of males and females*

While the main mass of our statistical data came from an examination of baskets made from the dried and shaped shells, a considerable number of individuals were identified as to sex. The arrays dealt with in this section consist of the scute counts of animals shipped to us alive, and of the advanced foetuses in our collection. The larger number of females is explained by the fact that our first interest was centered on the facts of development and hence we ordered only pregnant females. The number of individuals is, however, sufficiently large to furnish a basis of comparison between the sexes. Table 3 indicates the frequency distribution of the variates of the two sexes. It will be seen at a glance that the array of males here presented is decidedly more variable than that of the females. Part of the disparity may be due to the occurrence of one set of foetuses, all of which have a scute count of over 600; but even if we arbitrarily exclude this aberrant set we do not materially reduce the variability of the

male array The mean of the female array will be seen closely to approximate that of the whole collection, while that of the males is several scutes higher. It is our impression that a larger collection of males would place the mean at about 559 or 560, which would indicate a slightly higher center of frequency for males than for females. Although the mode of the two sexes

TABLE 3

*Showing distribution of frequency of scutes in the banded region of 146 females and 81 males*

FEMALES (146 INDIVIDUALS)				MALES (81 INDIVIDUALS)			
NUMBER OF SCUTES	FREQUENCY	NUMBER OF SCUTES	FREQUENCY	NUMBER OF SCUTES	FREQUENCY	NUMBER OF SCUTES	FREQUENCY
517	1	559	5	520	1	567	1
520	1	560	2	527	1	568	3
527	1	561	5	533	2	569	2
528	2	562	2	535	1	570	3
532	1	563	3	540	1	571	3
534	2	564	4	541	1	572	3
539	2	565	5	542	1	574	1
540	1	566	4	544	5	577	2
541	1	567	2	547	3	579	2
542	1	568	1	548	4	580	1
543	3	569	2	550	2	587	1
544	2	571	2	551	2	591	1
545	4	572	3	552	1	606	3
546	5	573	4	553	5	607	1
548	10	574	3	554	1	621	1
549	2	575	1	555	4		
550	4	577	1	558	1		
551	4	578	3	559	3		
552	4	581	2	560	1		
553	9	582	1	561	2		
554	3	583	1	562	2		
555	6	585	1	563	4		
556	5	589	1	564	1		
557	5	590	1	565	2		
558	6	596	1	566	1		
Mean = 557.2				Mean = 561.3 scutes			
Standard deviation=13.35 scutes				Standard deviation = 17.71 scutes			
Coefficient of variation=2.39 per cent				Coefficient of variation = 3.15 per cent			

[illegible]

given in convenient form the data, band for band. The arrays for the individual bands are considerably more satisfactory for purposes of seriation than that for the banded region as a whole; for the number of classes is comparatively small and consequently the number of counts sufficient to give a smooth curve without any grouping of variates. As a sample of the type of curve derived from a seriation of the variates of the individual bands let us take that of band 1 (fig. 2).

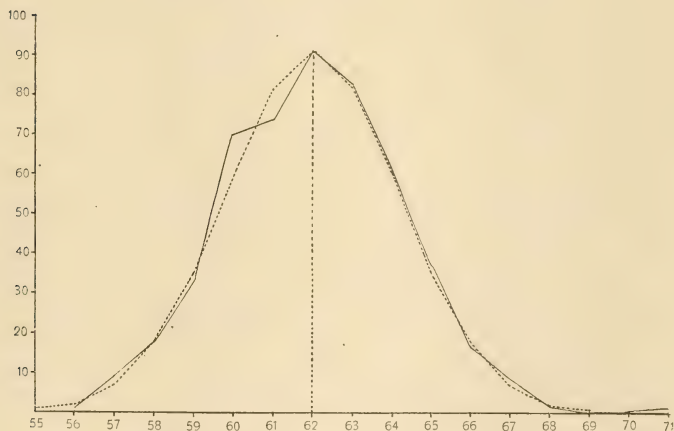


Fig. 2 Polygon of variation for the total number of scutes in the first band, as determined from a seriation of 508 individuals. Class range = 8 scutes. The solid line represents the observational and the broken line the theoretical normal curve.

It will be noted that the mean, mode and median coincide and that the observed and the calculated curve are almost identical. The curves for the other bands are of the same type as that shown for band 1. These observations would seem to indicate clearly enough that in the distribution of the elements of the integument into bands we have a pure chance process controlled by fairly simple mechanical laws.

In order that it may be clear that the sample of the species presented by the sets of foetuses studied is fairly representative, it would be well to compare the variation constants of the foetuses with those of the large sample of the species here dealt with. Table 5 furnishes the means of comparison. This table reveals to the student of biometry a number of interesting conditions not strictly pertinent to the present inquiry, but doubtless worth noting briefly:

1. With a few minor exceptions, all of which fall within the limits of probable error, there is a gradual decrease of the mean, mode and median from the first to the fourth band and then a gradual increase in these constants from the fifth to the ninth band.

2. The absolute variability, as indicated by the coefficient of variation is greater in each of the bands than in whole banded region.

3. The proportional range of variability is likewise greater for the individual bands than for the banded region as a whole.

4. The standard deviation may be considered for convenience to amount to two scutes. This will be worth remembering when we come to study the band correlation among the foetuses of the various sets.

For the convenience of the reader it seems well to consider the subject of hereditary control, as it is exhibited in connection with

TABLE 5  
*Showing the variation constants of the individual bands of 508 adults*

BANDS	MEAN	MEDIAN	MODE	STANDARD DEVIATION	COEFFICIENT OF VARIATION	RANGE IN PER CENT OF MEAN
1.....	62 $\pm 0.066$	62	62.0	2.223 $\pm 0.047$	3.59 $\pm 0.038$	24.19
2.....	60.55 $\pm 0.065$	61	61.9	2.217 $\pm 0.046$	3.66 $\pm 0.038$	23.3
3.....	60.44 $\pm 0.063$	60	61.76	2.12 $\pm 0.045$	3.51 $\pm 0.037$	26.46
4.....	60.23 $\pm 0.062$	60	60.92	2.08 $\pm 0.044$	3.45 $\pm 0.035$	24.9
5.....	61.16 $\pm 0.063$	61	61.64	2.11 $\pm 0.045$	3.46 $\pm 0.035$	24.54
6.....	61.85 $\pm 0.066$	62	62.3	2.23 $\pm 0.047$	3.61 $\pm 0.039$	29.1
7.....	63.09 $\pm 0.066$	63	62.82	2.22 $\pm 0.047$	3.52 $\pm 0.037$	20.6
8.....	64.43 $\pm 0.068$	64	63.14	2.24 $\pm 0.048$	3.49 $\pm 0.036$	26.39
9.....	64.44 $\pm 0.068$	64	63.12	2.2 $\pm 0.046$	3.57 $\pm 0.038$	23.27

the normal variability of the scutes in the banded region and in the individual bands, immediately following the variation data for the species, although it might appear to be more logical to complete the account of the conditions in the species before attacking that in the foetus sets.

*D. Fraternal correlation in the banded region as a whole; an index of the limits of hereditary control*

Having presented the facts of variation for the species, we are now in a position to investigate the conditions exhibited by the various 'fraternities,' which consist normally of 'identical quadruplets,' *i.e.*, of four individuals of the same sex enclosed in a common chorionic vesicle. We have in our possession at the present time about thirty sets of foetuses in a stage of development sufficiently advanced to permit of the determination of sex and of the accurate enumeration of scutes. Of these some few are of especial value on account of their more or less atypical conditions; a few others are characterized by an atypical number of foetuses. There are, however, ten sets of each sex in which the number of foetuses, the arrangement of bands, etc., is sufficiently normal to permit of statistical treatment. These twenty sets furnish the material for the present study of correlation and the limits of hereditary control. The enumeration of scutes may be relied upon to be accurate, since they were all counted independently by both of the writers and all points of discrepancy carefully examined and settled according to mutual judgment. A full tabulation of the scute counts of the twenty fraternities is presented in table 6.

As in our previous papers the four foetuses are numbered as follows:

Pair A	{	I. The lower individual of the right hand pair
		II. The upper individual of the right hand pair
Pair B	{	III. The upper individual of the left hand pair
		IV. The lower individual of the left hand pair

Since each of these sets of foetuses is derived from a single fertilized egg it should be possible to determine exactly to what

TABLE 6  
A—Female sets

SERIES	FOETUS AND MOTHER*	BANDS									TOTALS
		1	2	3	4	5	6	7	8	9	
2.....	I.....	60	62	60	60	60	63	63	66	63	557
	II.....	61	61	62	59	57	63	64	65	64	556
	III.....	60	60	59	59	61	64	63	63	64	553
	IV.....	60	59	61	60	63	62	60	64	62	551
23.....	I.....	60	61	58	60	60	60	61	65	66	551
	II.....	63	60	59	60	61	60	61	61	63	548
	III.....	64	61	58	60	61	61	61	65	63	554
	IV.....	61	62	59	61	60	63	64	66	62	558
95.....	I.....	59	57	58	63	62	63	63	67	61	553
	II.....	61	59	59	60	60	61	65	63	65	553
	III.....	62	58	60	60	61	62	64	67	64	558
	IV.....	62	60	59	60	63	62	63	64	65	558
98.....	I.....	63	61	63	60	61	63	63	65	67	566
	II.....	61	63	62	63	63	61	62	65	65	565
	III.....	61	62	61	61	61	61	65	64	66	562
	IV.....	63	60	62	61	62	61	63	65	68	565
	Mother.....	62	62	59	61	62	60	64	63	64	557
99.....	I.....	63	62	61	59	63	61	62	66	64	561
	II.....	63	61	63	61	61	62	64	64	65	564
	III.....	63	62	62	62	62	61	64	66	65	567
	IV.....	63	63	60	61	63	62	63	67	63	565
	Mother.....	65	64	60	62	65	62	64	66	66	574
119.....	I.....	62	60	62	61	61	60	64	62	62	554
	II.....	63	60	59	60	60	60.5	64	66	62	554.5
	III.....	61	60	58	60	60	61	62	63	65	550
	IV.....	63	59	60	59	61	60	61	63	62	548
	Mother.....	62	58	60	61	59	61	62	63	62	548
121.....	I.....	62	62	64	61	60	62	64	64	62	561
	II.....	61	62	59	62	63	62	63	67	66	565
	III.....	61	62	61	60	61	64	65	67	63	564
	IV.....	64	61	63	62	62	61	63	65	68	569
	Mother.....	60	59	61	61	63	64	65	67	65	565
122.....	I.....	62	60	61	60	60	60	59	61	61	544
	II.....	61	60	59	60	62	60	61	63	63	549
	III.....	61	61	61	59	59	59	61	62	63	546
	IV.....	62	62	60	61	60	58	59	64	62	548
	Mother.....	59	58	59	57	55	55	57	59	58	517
123.....	I.....	61	60	58	61	58	60	59	62	64	543
	II.....	63	62	61	57	58	61.5	58	63	61	544.5
	III.....	61	60	61	60	61	61	59	62	60	545
	IV.....	59	62	61	59	60	62	60	62	63	548
	Mother.....	58	61	59	63	61	60	63	65	63	553
127.....	I.....	61	58	57	57	60	62	62	61	63	541
	II.....	60	60	61	62	63	60	60	63	63	552
	III.....	61	59	60	60	59	61	61	65	62	548
	A rudimentary embryo lies between III and IV.....										
	IV.....	61	60	62	61	60	63	64	63	63	557
	Mother.....	58	60	60	61	61	62	64	65	64	555

\* In some of our first collections the mothers were not kept, and hence the scute counts on these cannot be given.

TABLE 6  
B—Male sets

SERIES	FOETUS AND MOTHER	BANDS									TOTALS
		1	2	3	4	5	6	7	8	9	
1	I.....	62	62	61	64	61	63	65	66	67	571
	II.....	62	62	63	64	64	62	65	65	67	574
	III.....	62	61	61	64	62	63	64	65	66	568
	IV.....	63	61	60	61	63	62	65	67	66	568
4	I.....	63	62	60	61	63	65	65	67	65	571
	II.....	64	64	63	60	61	63	64	67	65	571
	III.....	63	60	62	61	63	65	65	66	65	570
	IV.....	64	60	61	61	64	64	65	66	65	570
97	I.....	63	58	58	59	59	59	60	57	60	533
	II.....	61	59	56	56	59	58	57	60	61	527
	III.....	60	59	58	61	58	59	60	61	59	535
	IV.....	59	58	57	57	56	56	59	58	60	520
	Mother.....	58	56	60	56	57	61	60	60	59	527
112	I.....	62	64	61	60	62	60	60	63	63	555
	II.....	63	59	58	58	61	62	62	65	67	555
	III.....	60	59	58	58	60	61	60	65	67	548
	IV.....	61	60	62	60	59	60	60	63	63	548
	Mother.....	64	62	62	60	61	62	63	67	65	566
116	I.....	61	64	62	61	62	63	61	65	66	565
	II.....	63	61	62	61	62	64	63	63	66	565
	III.....	61	62	61	62	62	62	65	65	65	565
	IV.....	61	61	60	63	61	62	63	63	63	557
117	I.....	59	58	57	60	57	62	64	64	63	544
	II.....	60	62	61	56	57	57	62	64	63	542
	III.....	63	58	60	56	59	60	62	63	66	547
	IV.....	62	57	58	58	58	62	61	66	66	548
120	I.....	64	62	63	62	59	62	65	68	67	572
	II.....	66	62	64	63	63	63	65	67	66	579
	III.....	64	61	61	58	62	63	63	65	66	563
	IV.....	61	64	61	62	62	62	65	68	65	570
	Mother.....	63	60	59	60	60	59	63	65	66	555
134	I.....	64	62	60	59	59	60	62	63	62	551
	II.....	62	62	59	59	59	62	62	64	63	552
	III.....	64	64	60	60	61	60	63	65	63	560
	IV.....	64	60	60	62	60	62	62	64	65	559
	Mother.....	63	59	60	60	59	60	60	63	66	550
135	I.....	69	68	66	65	65	67	67	68	71	606
	II.....	68	64	66	63	68	69	67	71	70	606
	III.....	70	69	68	66	67	67	72	71	71	621
	IV.....	66	67	68	65.5	65	70	68	68	70	607.5
	Mother.....	63	60	63	62	63	65	64	66	67	573
138	I.....	62	61	59	60	61	60	64	63	63	553
	II.....	62	62	58	58	61	62	61	63	63	550
	III.....	61	62	60	59	61	62	63	62	64	554
	IV.....	61	58	60	61	59	61	63	64	64	551
	Mother.....	63	62	61	59	62	62	64	63	66	562

extent the definitive number of scutes is determined at the time of fertilization; for presumably, in so far as the four individuals of a set are alike, this similarity was determined before they became separate individuals, and, in so far as they differ, their differences are due to epigenetic factors operating after the separation. Should they prove to be exactly alike in the number and arrangement of scutes we would be warranted in claiming that hereditary control was absolute; if we do not find them exactly alike we may claim that hereditary control is not absolute but only partial. As an index of the strength of hereditary control we have decided to employ Galton's coefficient of correlation, a constant indicating exactly what per cent of complete correlation or of absolute hereditary control exists.

A short method for determining the coefficient of correlation, and one which seems especially devised for cases like the one in hand, was described by Harris ('09). This method appears to have been elaborated by Professor Pearson for use in cases of symmetrical correlation tables in which both variates have the same mean and standard deviation. The correlation table is made symmetrical by using each individual of each set first as a 'subject' and then as a 'relative.' In the case of our twenty sets of foetuses such a table requires over one hundred vertical and horizontal columns and would not be suitable for reproduction here. As a matter of fact it is scarcely necessary to make a correlation table in order to derive the desired constant. One need only determine the positive differences between the subject and relative classes and their frequency. In the present case we find the positive differences and arrange them as follows:

Differences:	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Frequency:	26	19	10	16	13	10	9	10	7	4	1	1	0	1	1	3	2

Multiplying the square of each difference by its frequency and adding the products, we obtain the sum of the squares of the differences [ $\sum v^2$ ]. Now since the negative differences are the same as the positive we may double the above sum and divide by the number of cases, 240, and thus obtain the standard deviation of the difference squared ( $\sigma v^2$ ), which is the only constant

not already determined that is needed for our calculation. The formula used is as follows:

$$r = \left( 1 - \frac{1}{2} \frac{\sigma v^2}{\sigma x^2} \right)$$

Substituting we get:  $r = \left( 1 - \frac{1}{2} \frac{33.5}{257.62} \right) = 0.9348.$

We can use this coefficient of correlation as an index of the strength of hereditary control and can say concretely that, so far as the total number of scutes in the banded region is concerned, the individual is predetermined within certain narrow limits, or up to 93.48 per cent of complete determination. Beyond that point the variations in the number of scutes are due to differences in epigenetic factors, whose nature we do not pretend to understand.

No such close correlation as this has been determined for any of the ordinary blood relationships. The closest of all blood relationships, namely the fraternal, is represented by a correlation constant of only 0.4, a fact familiar to all who have read their Galton. Even homotypical parts of the same individual are correlated only a little more closely than are brethren, a fact which leads Pearson to conclude that the fraternal relation is only a special case of homotyposis. In brief there appears to be no other inter-individualistic relationship so close as that which we have found to exist between the individuals of our sets of quadruplets. If we desire to find correlations comparable in closeness with that determined in our material, we must seek them among the closest of intra-individualistic relationships, such as that existing between the antimetrically paired organs of the same individual. As an example of such constants we may cite the correlation coefficient between the right and the left sides of the carapace of *Gelasimus*, which is 0.947, or that between the lengths of right and left meropodite of the first walking leg of the same species, which is 0.918. These constants are obviously of the same grade as that determined for the scutes of our quadruplets. From these facts we are doubtless justified in concluding that the four individuals of each of our sets is morphologically the equivalent of a

single individual and that we are dealing with a special case of intra-individual variation.

Were the phenomenon of specific polyembryony in need of any further support, the discovery that the degree of correlation among the foetuses of a set is the equivalent of that found to exist between the right and left sides of the same individual, would in itself constitute a demonstration of its validity.

TABLE 7

*Showing the variability of seven pairs of duplicate twins in terms of the coefficient of variation*

DESIGNATION OF TWINS	COEFFICIENT OF VARIATION
Weismann's .....	1.12
Vernon's I.....	0.22
Vernon's II.....	0.34
Wilder's I.....	1.43
Wilder's II.....	1.65
Wilder's III.....	1.52
Wilder's IV.....	1.17
Mean.....	1.06

In order to be able to institute a comparison between the strengths of hereditary control exhibited by armadillo quadruplets and human duplicate twins it becomes necessary to employ some method which does not involve the use of the coefficient of correlation, for the twin data is too scattering and diversified for our purposes. The usual method employed by the various writers for indicating the degree of resemblance between twins is that mentioned in the introduction, namely the per cent difference method, but this can be used only in the comparison of pairs. A better method for our purposes involves the determination of the coefficient of variation for each pair or set and an averaging of these constants. Table 7 gives the data on the seven pairs of human identical twins referred to above. In table 8 are recorded the coefficients of variation determined for the twenty sets of foetuses. It will be noted that the degree of resemblance among the quadruplets is, on the average, nearly twice as close as that of the twins. This is perhaps no more than we should expect, even

if we were certain of the origin of each pair of twins, for the twins have doubtless been much modified by post-natal environmental experience.

Incidentally it may be noted that the male sets exhibit a much higher absolute variability than the females sets, a fact which will be brought into discussion in a subsequent connection.

Perhaps a more equitable comparison between human twins and the armadillo sets would be instituted if we were to consider the quadruplet set as consisting of two pairs of twins. According to this method of comparison the difference between the two species is greatly increased, for the average of the forty pairs of armadillos is only 0.27 per cent, while that of the seven pairs of human twins is 1.62 per cent.

In concluding the present account of the strength of hereditary control as it appears to be exercised in the case of the total number of scutes in the banded region, it should be mentioned that further investigations now in progress, dealing with other regions of the armor and with certain dimensional variates, indicate

TABLE 8

*Showing the standard deviation of each of the sets of foetuses and indicating the comparative variability of male and female sets with respect to the total number of scutes in the nine bands.*

FEMALE SETS			MALE SETS		
SET	STANDARD DEVIATION (IN SCUTES)	COEFFICIENT OF VARIATION (IN PER CENT)	SET	STANDARD DEVIATION (IN SCUTES)	COEFFICIENT OF VARIATION (IN PER CENT)
2.....	2.38	0.43	1.....	2.48	0.43
23.....	3.69	0.66	4.....	0.5	0.08
95.....	2.5	0.45	97.....	5.86	1.10
98.....	1.5	0.26	112.....	3.5	0.63
99.....	2.16	0.38	116.....	3.46	0.61
119.....	2.59	0.46	117.....	2.38	0.43
121.....	2.86	0.50	120.....	5.70	0.99
122.....	1.92	0.35	134.....	4.02	0.72
123.....	1.87	0.34	135.....	6.36	1.04
127.....	5.85	1.06	138.....	1.56	0.28
Total	27.32	4.89		35.82	6.31
Mean	2.732	0.489		3.582	0.631

that the degree of correlation found to exist in the banded region has its parallel in other regions; in fact some of the results already obtained seem to indicate the existence of higher degrees of correlation than any yet determined.

*E. Fraternal correlation in the individual bands*

In one of our previous papers ('10) we had occasion to refer to the subject of pairing in the following words:

In this connection it should be mentioned that even where there is exact resemblance between the individuals of a pair in the total number of scutes in the nine bands of armor, there is no perfect correspondence with respect to individual rows. The resemblance in total numbers of scutes is, however, a matter of more importance than the exact manner of their arrangement into rows, which is a secondary process.

Further investigation has thrown light on this situation and we are now in a position to make a more satisfactory statement of the conditions referred to. By the application of statistical methods we have been able to compare the strength of hereditary control exercised over the total scute number and that over the arrangement of these scutes into bands. In order to do this it has been necessary to determine the correlation coefficient of each of the nine bands, using the same method which was applied to the whole banded region. The method pursued is illustrated in the case of band 1 (table 9). The same method of procedure was carried out for each of the nine bands and the data and results are seen in concise tabular form in table 10.

Although the average coefficient of correlation for the individual bands is high as compared with that found for any relation other than an intra-individualistic one, it is decidedly low as compared with that shown to exist in the case of the total number of scutes in the whole region. This would seem to indicate that there is here a much wider scope for the operation of epigenetic factors. Evidently the alignment of scutes to produce the bands is, to a large extent, a mechanical process, involving a certain amount of shifting due to pressure, etc. In the earliest stages of scute formation it is probable that the primordia of these elements are arranged somewhat after the fashion seen in the abdominal



TABLE 10

*Showing coefficients of correlation and other constants in each of the nine bands of armor*

BAND	MEAN	$\sigma X^2$	$\sigma Y^2$	COEFFICIENT OF CORRELATION
1.....	62.16	4.090	3.10	0.6201
2.....	61.03	4.712	3.94	0.5820
3.....	60.05	5.887	3.80	0.6769
4.....	60.45	3.972	3.93	0.5051
5.....	60.97	4.828	3.41	0.6469
6.....	61.73	4.970	2.52	0.7458
7.....	62.70	5.735	3.30	0.7123
8.....	64.40	5.155	3.75	0.6363
9.....	64.22	5.875	3.66	0.6880
Mean .....				0.6459

region, where the rows are far from straight and where it is not always possible to assign scutes to their respective rows. At such a stage in the development of the banded region we may assume that the ultimate position of certain scutes is determined only in a general way, and whether or not they come to be aligned with one or the other of two adjacent bands is determined by factors beyond the limits of hereditary control. Considering that the scutes are not only determined rather sharply for the whole region, but that, within this region, their primordia would also be regionally distributed under the influence of hereditary control, the coefficient of correlation is no larger than we should expect. But the correlation is so low as to preclude the possibility of any strong hereditary control being exercised over the assignment of scutes to particular bands. This conclusion is in close harmony with that expressed by Wilder in connection with his studies of friction ridge patterns in human twins, and which is quoted in the introduction to the present paper. We might well ask with him: "*Have we then arrived at the limit of hereditary control of the pre-determining mechanism beyond which mechanical laws are alone operative?*" Perhaps we are in a position to give with somewhat greater assurance than was Wilder, an affirmative answer to the question.

## ATYPICAL VARIATION IN THE INDIVIDUAL ELEMENTS AND IN THE BANDS

*A. Scute 'abnormalities' in the species; their distribution and frequency*

(1). *Double scutes.* In the individual scutes and in the bands certain so-called 'abnormalities' frequently occur, and while these may be but the expression of teratological phenomena, yet we believe that some of them at least are the result of more deeply seated factors and have a real phylogenetic significance, and consequently are worthy of consideration in a paper on variation and heredity. We shall speak of them as atypical variations.

The atypical variation of the scutes is expressed in at least three different ways, one of the most frequent of which we shall designate as the 'double scute.' In this type two contiguous primary scutes are fused along their adjacent sides, and the double structure thus formed has five or six hairs at the free or posterior end (figs. 6a, 7a, 8a). Evidently there is a suppression of two or three hairs consequent upon this union. All stages of fusion have been observed in our material, and in the figures just referred to is seen a series of three, taken from one specimen showing different degrees of the process. The nature of the origin of these double scutes is made clear when the region affected is examined from the under side, where the double bony element is always clearly expressed (figs. 6b, 7b, 8b). Sometimes the bony elements are of equal size and occupy the full width of the band, but more frequently one of the plates is greatly reduced, as if there had been a tendency to crowd it out (fig. 8b).

The double scutes occur in about ten per cent of the animals—or to be more exact, in a total of 516 individuals examined for this particular point 51 showed double scutes. Ordinarily there is but one of these to an animal, but four exceptions to this have been found, as follows: one with four double scutes, one with three, and two with two each.

For the purpose of locating exactly the various scutes, we have chosen to begin their enumeration always on the left margin of the band. Thus scute '10' of band 5 would be the tenth scute

counting from the left margin of this band. Since it is obvious that the double scute is the product of what was originally two distinct elements, it has been given the value of two in our collected data, though we designate it by the number corresponding to its first or left-hand element. This point can be made clear by a reference to the numbering in figs. 6 to 10.

The double scutes are generally distributed over the bands as can be seen in table 11. There is, however, a tendency for them

TABLE 11  
*Showing distribution of 'double scutes'*

BANDS	DISTRIBUTION IN BANDS	LATERAL DISTRIBUTION		
		Left	Middle	Right
1.....	2, 4, 7, 11, 23, 24, 31, 42	4	3	1
2.....	10, 10, 14, 15, 27, 29, 57	4	2	1
3.....	12, 25, 27, 29, 41, 53, 57, 59	1	3	4
4.....	3, 19, 19, 23, 54, 54, 55, 60	3	1	4
5.....	2, 2, 2, 32, 44, 53, 57	3	1	3
6.....	2, 5, 6, 25, 27, 28, 56, 57, 58	3	3	3
7.....	3, 12, 24, 50	2	1	1
8.....	2, 15, 36	2	1	0
9.....	36, 65	0	1	1
Totals.....	56 cases	22	16	18

NOTE, in the left-hand half of the table the numbers in the horizontal rows lying opposite the bands designate the first elements upon which the double scutes fall. Thus in the first band eight animals had these scutes, falling on elements 2, 4, 7, etc., respectively.

to be localized in the anterior two-thirds of the banded region, or in bands one to six. Perhaps of more importance is their lateral distribution, which can be shown by dividing each band into three equal parts—two of which represent the left and right thirds and one the middle third—and determining the number of double scutes falling within each of these general divisions. The data thus collected is shown in the right half of the table, from which it is clear that there is a tendency for these scutes to be localized toward the margins of the armor. They fall most frequently

on scute '2' (six cases) on the left, and scute '57' (four cases) on the right. However, it should be pointed out that there are but four cases of exact coincidence (one involving three specimens) by which we mean double scutes falling at the same point in the same band in two or more individuals.

(2). *Incomplete scutes.* The second type of atypical scute variation is just the opposite to that of the preceding, in that it is probably the product of a splitting of what was originally a single scute, and results in the formation of an incomplete element. This type appears so rarely that not very much importance can be attached to it. In the 516 specimens examined only three cases of incomplete scutes were found. These occur as follows: Specimen one, a female, between scutes 52 and 54 of band 6 (the small plate is counted as a whole one); specimen two, a shell, between scutes 14 and 16 of band 8; specimen three, also a shell, between scutes 56 and 58 of band 5. The first and second of these are sketched in figs. 9 and 10, respectively. The most interesting point brought out is that in each case one of the scutes lying adjacent to the incomplete one has but three hairs instead of the typical four, while the small scute has in each case but a single hair associated with it. The presence of three hairs in an adjacent scute would not alone be cogent proof that the primordium of such a scute had split to give rise to the incomplete element, as will be evident when the next section of the paper is reached. However, all of the other relations in these specimens point toward a fission process.

(3). *Three-hair type of scute.* The third type of atypical variation is perhaps the most fundamental of all, because it involves a distinct change in the morphological unit of the scute. In this type the bony plate underlying the scute has but three hairs at its posterior end instead of the customary four. It was first detected in a shell which had an unusually high number of scutes in the bands; in fact the one representing the most extreme case of high numbers that we have so far found. When the shell is first observed the attention is at once attracted by the narrowness of the scutes, and upon examining them the interesting fact is revealed that the majority show the three-hair type (fig. 22).

We have studied this shell in considerable detail and have compiled the data secured in table 12. Here it will be noted that 391 of the 625 scutes have but three hairs each, and furthermore that there is a strong tendency for the 3's to be distributed on the lateral parts of the shield. However, the most striking fact revealed in the table is indicated in the fourth column, where the total number of hairs for each band is given. Bands 2 and 8, which show the extremes in scute variation, have exactly the same number of hairs, while the two extremes in hair variation, bands 7 and 9, are but six hairs apart in their totals.

TABLE 12

*Shell 2, with 625 scutes showing distribution of 3-hair and 4-hair scutes*

BANDS	DISTRIBUTION OF 3'S AND 4'S IN BANDS			LATERAL DISTRIBUTION OF 3'S		
	3-Hair Type	4-Hair Type	Number of Hairs	Left	Middle	Right
1.....	49	22	235	19	16	14
2.....	34	33	234	14	12	8
3.....	37	31	235	18	5	14
4.....	38	30	234	21	3	14
5.....	38	30	234	19	6	13
6.....	49	22	235	23	4	22
7.....	41	27	231	18	7	16
8.....	58	15	234	21	16	21
9.....	47	24	237	23	8	16
Totals.....	391	234	2109	176	77	138

The question naturally arises: Can the high number of scutes exhibited by this specimen be accounted for by the fact that so many of them are of the three-hair type? Undoubtedly it can, but the affirmative answer necessitates the assumption that the integumentary primordium out of which each band arises contains a definite number of hair follicles, which are later distributed into groups of 3's and 4's according to the propensities of the formative scutes. It is evident upon this assumption that a tendency for the scutes to form about groups of four hairs would result in the production of a shell having a low number of scutes, while

a tendency to form about groups of three would produce just the opposite result. A reference to table 13, in which the total counts on five specimens are recorded, indicates that, although the animals may vary considerably in the total number of scutes, yet

TABLE 13

*Showing proportion of 3-hair and 4-hair scutes in a small sample of the species*

SPECIMEN	NUMBER OF SCUTES	3-HAIR TYPE	4-HAIR TYPE	NUMBER OF HAIRS	LEFT	MIDDLE	RIGHT
Shell 2.....	625	391	234	2109	176	77	138
♀ 221 .....	577	159	418	2149	70	29	60
♀ 223 .....	545	77	468	2103	33	9	35
♀ 217 .....	534	19	515	2110	7	2	10
♀ 204 .....	529	26	503	2091	13	0	13

there is very little difference in their total hair counts. In the two extreme cases, shell 2 and female 204, there is a difference of 96 scutes, but in total hair counts they vary by only 18 points, which is a very inconsiderable difference when one considers that there are between three and four times as many hairs as scutes. Undoubtedly data on a larger number of animals would reveal a much greater variation; but the fact remains that, however variable the species may be with respect to the scutes (and their associated plates), it is comparatively stable with respect to the hairs of a given region. A statistical study of the hairs might be made, but their enumeration is difficult; for, on the one hand, not a few of the adults possess armors from which many of the hairs have been lost by abrasions through contact with rocky dens, and on the other hand, even advanced fetuses do not have hairs sufficiently developed to allow a census of them being taken.

For this and other reasons it has seemed best to confine our studies to the variability and heredity of the scutes, and not to attempt a compilation of statistics on hair counts, which at best could be only imperfect. It is perhaps sufficient then merely to have indicated the line along which the evolution of the species is apparently directed. The fact that in all of the regions showing a primitive condition of the scutes, as in the case of the belly,

the hair groups are larger than in the more highly differentiated region like that of the bands, is *prima facie* evidence that here the direction of evolution is from the four-hair to the three-hair type, with a consequent increase in the total number of scutes.

These high numbered specimens with their many scutes of the three-hair type are of further interest because they are probably to be regarded as mutations, in the sense that they represent discontinuous variations. This in part can be made clear by an inspection of table 1. At the lower end of the series therein represented the specimens occur with a frequency sufficient to be explained on the basis of an ordinary fluctuating variability, and this is also true of the other end of the series up to the specimen with 596 scutes; but beyond this point specimens are of rare occurrence and consequently are separated by wide gaps. However, neither the rarity of their occurrence nor their wide separation is sufficient to place such specimens in the category of mutations, because there is always the possibility that a larger number of the species would furnish variates enough to fill up the gaps. If we would therefore explain these as saltations we must look to another source of information, namely, to their behavior in inheritance. This topic will receive attention in a succeeding section.

#### *B. Hereditary control in connection with scute 'abnormalities'*

(1). *Double scutes.* Attention has been called to the comparative rarity of double scutes in the species and to the fact that in only a very few cases do double elements fall in the same place in the same band. It is rarely the case even that the same general region of a band of more than two specimens shows the peculiarity in question. When, therefore, we find in as many as three out of four members of a set of fetuses a double scute in almost precisely the same locality, we are forced to the conviction that even these elements, which might be defined in the words of Galton as '*the minutest biological units,*' are predetermined in the undivided germ cell.

Two sets of fetuses show the conditions most clearly and they are described in detail below:

*Set 121 (Mother without any double scutes)*

- Foetus I. No double scutes  
Foetus II. In band 2, scute 29, double  
Foetus III. In band 2, scute 28, double  
Foetus IV. In band 3, scute 28, double

It is to be noted that in three of the four individuals the double element occurs practically in the same spot. In view of the fact that, even in perfectly normal sets, the number of scutes in the same row varies widely among the individuals, it would hardly be expected that a predetermined doubling could strike the same numbered scute in any two members of a set. The regional hereditary control in this case is therefore somewhat more accurate than we would expect to find it, in view of the laxity of hereditary control in the matter of the arrangement of scutes into bands. The fact that in one of the foetuses the double scute occurs on the third band, while in two other foetuses the doubling affects the same or contiguous scute of the second band, only goes to bear out the idea that the process of scute alignment is to a large extent a mechanical process, involving the shifting backward or forward of the individual primordia under the influence of pressures. It is only to be expected then, in the light of these considerations, that we should find a case now and then where a readily recognizable element like a double scute should indicate by its position that it may have been shifted from one row to another.

On account of the small extent of its 'abnormalities' this set has been used in the study of correlation (table 6, A) as well as for an example of a peculiar type of band arrangement, (p. 874).

*Set 123 (Mother without atypical scutes of any kind)*

- Foetus I. In the last row of the scapular shield, scute 30 is double; scute 2 of band 2, double; scute 10 of band 3, double  
Foetus II. In the last scapular row scute 28, double; an incomplete scute like that shown in fig. 9, occurs in band 6 between scutes 2 and 3  
Foetus III. In the last scapular band scute 14, double; scute 49 of band 6, double  
Foetus IV. Scute 31 of band 4, double

In this set the following points are noteworthy:

1. In none of the specimens examined in the statistical study of the frequency and distribution of double scutes was a case of doubling found in the last scapular row; hence the likelihood of the conditions described being due to coincidence is extremely remote.

2. The location of the double scute is evidently not so rigidly defined as in the former case, since the double scute in foetus III is rather far separated in position from that of either of the other foetuses. In the two individuals of the natural pair A, of the left hand side (right in the figure), however, the position of the double scute is almost precisely the same. One can detect the difference in position only by counting the scutes. Careful examination of the two right hand individuals in fig. 24 will make this clear. In the subsequent discussion of the phenomenon of pairing this circumstance will receive further attention.

3. It is very unusual, as was indicated in the general discussion of scute 'abnormalities,' for an individual to have more than one double scute. When, therefore, three of the four quadruplets have two or more double scutes and the other has one we are inclined to suspect that they are all predetermined.

4. In this and the last set the 'three-to-one' proportion is shown in several ways: (a) In set 121 three show a double scute and one lacks it; (b) in set 123 three have the scapular double scute and one lacks it; (c) in the same set one of the four has three double scutes, one has only one double scute, and one of the four has an incomplete scute in place of a double scute. Many other cases of a similar kind are noted in connection with both normal and atypical sets.

The following points although possibly of no real significance should be noted: (a) In foetus IV, the adjacent of foetus I, occurs a double scute in the same position in its band as that which occurs in the scapular region of foetus I, but four bands posterior to the latter. (b) In foetus II occurs an incomplete scute occupying the same position in its band as does one of the double scutes of foetus I, but situated four bands posterior to the latter. (c) In foetus III a double scute of the sixth band is in position almost

exactly the 'mirror image' of the double scute in the third band of foetus I.

These facts may indicate various degrees of imperfect hereditary control in connection with the localization of these minute 'abnormalities,' but if this be the case we are dealing with a state of affairs too complex to admit of solution with the present material.

There are several other sets which exhibit scute 'abnormalities.' These may for convenience be brought together into a compact table (14). Here the number of the double or incomplete elements in the various columns refers to the position of each in the band; 'D' indicates double scute and 'S' incomplete or split scute:

Although in the members of these sets the double or incomplete elements would appear to occur almost at random, so far as their position within the nine bands is concerned, there are some cases in which, within a given pair at least, the hereditary control is fairly obvious. Table 14 further indicates that a close morphological relationship exists between the two types of 'abnormality' and that they may readily be imagined to have a common hered-

TABLE 14

*Showing the distribution of double and split scutes in the 20 sets of foetuses*

SET	FOETUS	BAND								
		1	2	3	4	5	6	7	8	9
2.....	I.....		15D							
	II.....	58D								
	IV.....					37D				
99.....	III.....			41D						
	IV.....				46D					
116.....	I.....	9D								
	II.....				25D					
	III.....							9D		
119.....	II.....						3S			
127.....	I.....								13D	
135.....	I.....		25D			54D				
	II.....				60D					
	IV.....				60S					
13S.....	III.....	11D								

itary basis. It would seem to be profitless to attempt any detailed analysis of the conditions in the sets tabulated, but there are some points worth study that have not been mentioned.

Every case in which two or more individuals show a scute 'abnormality' is almost undoubtedly a case involving some phase of hereditary control, for on the basis of chance there would be very few sets more than one individual of which would have a double scute.

In concluding this account of the hereditary control of double scutes attention might be called to the fact that in this connection we have excellent material for testing Galton's "minutest biological unit capable of hereditary transmission." Whether or not these minute peculiarities of the scutes are directly inherited from the immediate parents we cannot at present determine, but we are certain that the conditions are blastogenic in the sense that they are predetermined in the fertilized egg. If this be the equivalent of hereditary transmission we may rightly claim to have found just the sort of unit that Galton was looking for. It seems unlikely that any smaller unit could be predetermined.

(2). *Three-hair type of scute.* In only one set of foetuses (set 135) have we found that peculiar condition described as a possible discontinuous variation or mutation. Although we are unable to count the hair primordia in the foetuses, we are confident that the great majority of them are of the three-hair type. This assumption is justified by the fact that the scutes have the same narrow appearance that is seen in adults in which the three-hair type prevails, and by the additional circumstance that we have never found an individual with over 600 scutes in which the three-hair type of scute did not largely predominate. As an additional piece of evidence in favor of the idea that the condition in question is a mutation, it seems highly probable that it is inherited in the exclusive fashion and is dominant. If we suppose that the foetuses show a blend between the mother, an individual with 573 scutes, and the unknown father, it would necessitate the positing of a male parent with a scute number far in excess of any we have found to occur in the species. That the lower scute numbers, which are made up from shells showing comparatively few three-

hair scutes, are inherited in the blended fashion is shown by a comparison of the counts of the mothers and those of the foetuses. In nearly every case the latter show marked divergences from the former, which would seem to indicate a total lack of exclusiveness about the inheritance.

Attention might also be called to the fact that in this set three individuals have almost identically the same number of scutes while the fourth has decidedly more.

*C. Band 'abnormalities' in the species; their distribution and frequency*

The atypical variations in the bands consist primarily of extra or supernumerary bands or parts of bands, and show a rather marked degree of regularity in that they occur repeatedly in about the same regions of the armor. They are found most frequently in the first or second band, or in both, and may occasionally be seen in the region of the eighth or ninth band, but rarely appear in bands three to seven. Their frequency has been determined from a study of 1768 specimens, in which 60 are abnormal, or about 3.4 per cent of the total number of individuals examined.

On account of the apparent nature of their origin, as well as for convenience in description, we have chosen to consider these 'abnormalities' under three headings, as follows: (1) Fusions, in which parts of two bands have united to form a single structure; (2) Splittings, in which a series of elements of a band have divided transversely to produce a double row; (3) Additions, in which a band or part of band, either from the scapular or pelvic shield, has been added to the thoraco-lumbar shield, thereby increasing the banded region, and in case of the contribution of an entire band, producing a ten-banded animal.

(1). *Fusions*. This kind of variation is found in 31 of the 60 'abnormal' specimens, or in more than fifty per cent of the cases. It usually expresses itself in one of two ways. In one type two adjacent bands have their scutes at one side of the armor united to form a single structure. This type may be spoken of as 'unilateral,' in contrast to the second or 'bilateral' type, in which the

fusion of the two bands occurs on both sides of the armor. A very good example of this latter type is shown in fig. 11 in which seven scutes are involved on the left side and four on the right.

The unilateral type occurs 25 times in the 31 cases of fusion, and appears 15 times on the left side and 10 on the right. It is distributed as follows among the several bands: 18 times between bands 1 and 2; 3 times between bands 8 and 9; and once each between bands 2 and 3, 4 and 5, 5 and 6, 6 and 7. In almost every case of unilateral fusion the scutes at the extreme lateral margins of the bands are involved, and in only a few specimens is it situated well within the margin (e.g., in fig. 18).

The six remaining cases of fusion are all of the bilateral type, and in each instance the two fusions are practically symmetrical, both as to position and extent. This is true even when they are situated toward the median line of the armor. All the bilateral fusions observed are between bands one and two.

(2). *Splittings*. The second kind of atypical band variation is the splitting, which as already stated consists of a transverse division of the elements to give rise to two bands. It is not always an easy matter to determine, especially in complex cases where two bands seem to be greatly confused, whether we are dealing with a fusion or a splitting; but a rather safe, though not universal, rule to follow is to count the elements in each row of the double regions, and if these be equal in numbers it is safe to decide that one is dealing with a splitting (fig. 12).

Using this as our principal method of determination we have classified sixteen specimens as belonging to this group. Five of these are unilateral (three on the left and two on the right) and eleven bilateral. One of the most striking features of the latter type is the strong tendency to be almost bilaterally symmetrical. For example in fig. 12 is shown one in which the splitting begins in the seventh scute on each side, and the specimen fails to show symmetry in its bilaterality only in having the splitting slightly less extensive on the right than on the left side. Another point worthy of note is the fact that splittings are confined almost entirely to the first band; thirteen of the fifteen cases occurring here.

The fourteenth and fifteenth cases appear in the sixth and ninth bands, respectively.

Several of the cases of splitting are rather complicated, but not to such an extent as to render their classification uncertain. One of these is shown in fig. 13, and it will be noted that while the 'abnormality' is clearly of the bilateral type, yet it is slightly complicated by having an additional splitting just to the right of the left-hand split.

(3). *Additions.* The final class of band variations is additions, by which is meant the adding of a band or part of band from either of the shields lying adjacent to the banded region. Six cases of this type have been observed, and five of these are from the scapular shield. An excellent example of this type is seen in fig. 19. The right half of the posterior row of scutes has dropped down from the shield and forms a perfect band on this side of the armor. One can follow the scutes of the added half-band across to the left side where they clearly form the posterior row of the scapular shield, so no question can exist regarding the origin of such 'abnormalities.'

It is easy to imagine how a completion of the dropping would result in adding an entire new band to the banded region, and the four or five cases of ten-banded animals that we have observed may have acquired their extra band in this manner. However, it is possible that additions may take place from the pelvic shield; in fact, our sixth case of additions has probably come to exist in this way, because the pelvic shield of this particular specimen is foreshortened. It should be noted also that a ten-banded animal could be produced by a complete splitting of one of the nine regular bands.

In four of the seven cases of band variations, not classified in the above groups, not sufficient data were taken to permit an exact determination of their characters; they are merely recorded as being 'abnormal.' Two of the remaining three are almost exactly the same, except that one is in the first band and the other in the ninth. The latter is sketched in fig. 14. The 'abnormality' is located approximately in the middle of the band, and consists of a region of four double scutes. At first one would be inclined

to suggest that it had been produced by a splitting of four scutes, but upon a closer inspection it will be seen that the upper row is closely associated with the left half of the band, and the lower row with the right half. In the light of the 'theory of conrescence' one is tempted to suggest that it has been brought about through a failure of the embryonic primordium to affect a perfect meeting in this region, and consequently the inner ends of the two half-bands have slipped past each other for a short distance.

In this connection attention should be called to a certain rather rare atypical condition which sometimes appears either in the ninth band or in the first row of the pelvic shield. The peculiarity consists of a 'jog' at the middle point of the band or row, and in all probability has come about in a manner like that suggested just above. A specimen showing this condition in the first row of the pelvic shield is seen in fig. 23.

The final 'abnormality' to be considered is shown in fig. 15. It really is a double peculiarity, in that both the eighth and ninth bands are involved. In the eighth band there are three small primary scutes lying just anterior to numbers 53-56 of the main scutes of the band. These small scutes do not affect the bony plates and must therefore be looked upon as very rudimentary in character. In the ninth band a somewhat similar 'abnormality' is seen, lying at scutes 52-56 of this band. It consists of four small scutes, which however affect the bony plates, as can be seen in the sketch of the under surface of this region of the band (fig. 15 *b*). It cannot be said to be a splitting because there are five plates in the lower or main row and only four above.

In concluding this section of the paper two facts brought out in the foregoing account should receive especial attention, because of their direct bearing on what is to follow. (1) In all those cases of atypical variations that we have designated as bilateral there is a very strong tendency toward symmetry in the affected regions both in position and extent. This is particularly clear in specimens classed as splittings, as the citation of a few cases will make evident: (a) In shell no. 8 band 1 has 57 scutes, and the two splittings occur in scutes 6-14 on the left and 44-51 on the right—were the right-hand split in scutes 44-52 instead of

44-51, it would be a perfect example of bilateral symmetry; (b) in shell no. 21, in which band 1 has 59 scutes, the splits are in scutes 8-13 and 46-52—here again a change of one point on the right side would make a bilaterally symmetrical 'abnormality'; and (c) in shell no. 13 there are 63 scutes in band 1, and the splits are in scutes 4-16 and 48-59— a close approximation to symmetry. These conditions remind one of Wilder's results in duplicate twins, already referred to in the introduction. (2) The second point to be emphasized is this, that although the atypical variations in the bands show a marked degree of regularity in occurring so frequently in the first and second bands, yet they display a great diversity in the fact that scarcely any two of them are exactly alike. This diversity for the species is striking when considered in the light of the fact that in 1768 specimens examined but two sets of coincidences have been found, and these occur in the simplest type of 'abnormality.' Since many of the shells upon which these data were taken are from the same locality it is not improbable that some of these cases are either brothers or sisters, and not coincidences. The fact that only two sets of these supposed cases of coincidences, and these of the simplest type (very slight fusions), have been found in 1768 individuals will serve to emphasize the importance of fraternal correlation as brought out in a subsequent section of the paper.

*D. Hereditary control in connection with band 'abnormalities'*

Considering the comparative rarity of band 'abnormalities' in the species we have been fortunate to secure a collection of sets of fetuses, among which appear examples of practically all of the types of malformation described above. As in the case of meristic variation in the normal scutes and in the matter of double scutes the precision of hereditary control is much more marked in some cases than in others; in some the conditions are fairly simple and in others highly complex. The sets are described in detail and discussed separately, in so far as the special conditions of each case are involved. The general significance of many of the observations can be discussed to advantage only after all

the data has been presented and the underlying problems presented for discussion.

*Set 64 (Mother normal).* Foetus 1 appears at first sight to be perfectly normal, in so far as band arrangements are concerned; but examination reveals the presence of ten full bands. In view of the fact that all of the other members of the set show more or less extensive regions of splitting in the first band we are forced to conclude that the extra band in this individual has been produced by a process of band splitting carried to a completion.

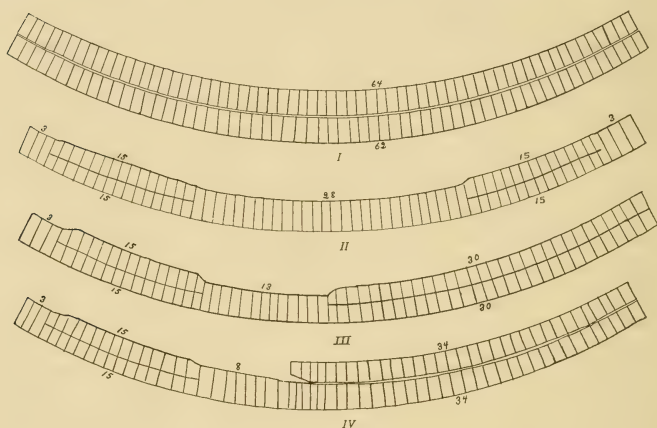


Fig. 3 Diagrams of the 'abnormal' bands in the four fetuses from female no. 64. In this as in the two succeeding figures, the Roman numerals I-IV refer to the individual embryos from which the sketches were made, while the arabic indicate the number of scutes in each of the regions to which they are adjacent.

This is really only a bilateral expression of the condition seen in the right hand half of the first band of fetuses III and IV. That there are 64 scutes in the first half-band and only 62 in the second might seem to militate against the idea of splitting, but there occur in our collection several undoubted cases of splitting where the number of scutes in the two series produced by the split are unequal. The split first band of foetus 1 is shown in fig. 3, 1.

Foetus II shows a condition entirely different from that described for its partner, namely a bilaterally symmetrical, regional splitting of the first band, beginning four scutes from the margin on each side and involving 15 double rows of scutes in each case. The condition is diagrammatically shown in fig. 3, II.

Foetus III exhibits a decidedly asymmetrical regional splitting of the first band, being a mixture of the two pure types shown in foetuses I and II. The left half of the band is identical with that of foetus II, while the right half is the duplicate of that in foetus I (fig. 3, III).

Foetus IV is identical with foetus III, in so far as the splitting is concerned, but shows in addition to the latter a fusion of the anterior row of the completely split half with the opposite half of the last scapular row of scutes. This condition has been observed in the shells of several adults and has been interpreted as a case of the incipient addition of a band to the banded region by means of a 'drop-down' from the scapular shield. The present condition could hardly be interpreted in that way in view of our knowledge of the conditions seen in the other members of the set. It would appear that we have here a case of an epigenetic process, involving a secondary fusion of two unrelated half bands, the right half of the first band and the left half of the last scapular row (fig. 3, IV).

This is in some ways the most extraordinary set in the collection and suggests a number of theoretical considerations for general discussion. The main points that should be noted while the facts are fresh in mind are as follows:

1. The splitting process involving the first band occurs in all members of the set, which would indicate that this much was predetermined.

2. The regional splitting involves in all four cases (twice in foetus II) exactly the same number of scutes, 15 in each case; and these are located precisely the same distance from the margin every time. The precision of hereditary control is, in this regard, nothing short of marvelous, since it is perfect.

3. There are evidently two distinct expressions of the splitting tendency, the complete and the incomplete. The distribution of

the two types is quite impartial in so far as their frequency is concerned, for, out of the possible eight lateral halves of the four foetuses, four are occupied by each type. It would appear from this that each was equally strongly predetermined; but the exercise of hereditary control in the distribution of the two types among the four foetuses is somewhat haphazard and reminds one strongly of a pure chance combination of two elements selected in pairs, like, for example, the Mendelian ratio of F 2's, D-2Dr-r.

4. Apart from the 'secondary' fusion of one of the split half bands with the scapular shield, the members of the natural pair B (III and IV) are strikingly identical. Evidently hereditary control within the pair is more accurate than in the whole set. The explanation of this condition must be discussed in the subsequent section on pairing.

*Set 96 (Mother normal).* This case is somewhat simpler than the last in that it involves only one type of 'abnormality,' namely a simple fusion of the first two bands. We have decided to call the condition a fusion, because, including the two which are united, there are only nine bands. The strictly marginal character of the band unions would also serve to indicate a fusion, for we have found no cases of incipient marginal splitting in our examination of adult shells.

Foetus I shows a unilateral fusion of comparatively slight extent, involving only 5 scutes and confined to the right side. There are 57 free scutes in band 1 and 58 in band 2 (fig. 4, I).

Foetus II shows a bilateral fusion of small extent, involving 7 scutes on the right and 4 on the left. There are 51 free scutes in band 1 and 49 in band 2 (fig. 4, II).

Foetus III shows an extensive unilateral fusion, involving 21 scutes and confined to the right side. There are 40 free scutes in band 1 and 39 in band 2 (fig. 4, III).

Foetus IV shows the same condition as does foetus III except that there are 19 fused scutes instead of 21. There are 41 free scutes in each of the first two bands (fig. 4, IV).

The following points may be noted:

1. The fusion in the first two bands occurs in all individuals but differs in its extent and in the unilaterality or bilaterality of its

expression. Evidently a marginal fusion of bands 1 and 2 is predetermined, but here the predetermining influence ceases to operate and epigenetic factors of some sort determine whether the character shall have a unilateral or a bilateral expression and to what extent the fusion is to be carried in each case. It will be noted that hereditary control is much less precise in this case than in set 64.

2. The 'three-to-one' proportion once more presents itself in that three members of the set have the fusion on the right side only, while one has it on both sides.

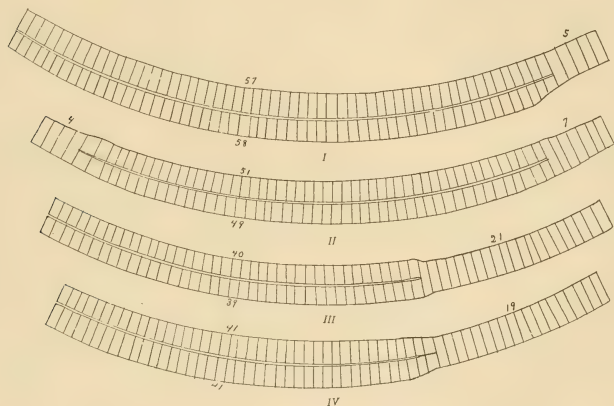


Fig. 4 Diagrams of the 'abnormal' bands of the four fetuses from female no. 96.

3. There is a distinct pairing on the basis of the extent of the fusion, pair A (I and II) showing the character in a much less extensive form than pair B (III and IV). Within the pairs the resemblance is very close.

*Set 101 (Mother normal).* The conditions seen here are in many respects equivalent to those described for set 64. All four fetuses show a rather advanced stage of splitting in the first band. At first we thought that fetuses III and IV were quite

normal, but further examination reveals our mistake, for each of these two individuals possesses ten well defined bands. The condition seen in one of the fetuses is so obviously a splitting that we are compelled to interpret the extra band in each of these fetuses as having been produced by a complete splitting of the first band.

Foetus I shows an extensive bilaterally symmetrical and somewhat complex regional splitting of the first band. On both sides, beginning 6 scutes from the margin, there occurs a splitting involving 9 scutes, and in the central part of the band there is another

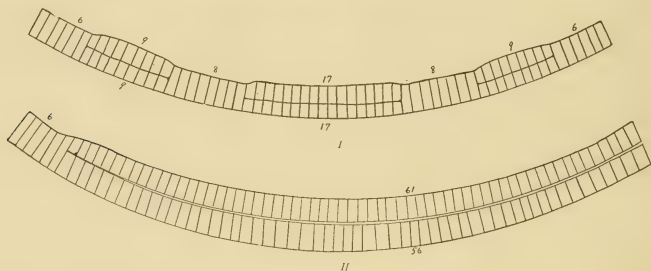


Fig. 5 Diagrams of the 'abnormal' bands of fetuses I and II from female no. 101.

split region of 17 scutes. Separating the median from the two lateral splittings are paired unsplit regions, each involving 8 scutes. In the ninth band there is another band peculiarity, consisting of a 'jog' in the middle of the band similar to that shown in fig. 23. There are 34 scutes to the left of the break and 31 to the right. The region involving the fusion is illustrated diagrammatically in fig. 5, I.

Foetus II shows a more advanced stage of the splitting in that the process has gone on to completion on the right side and has extended to within 6 scutes of the margin on the left side (fig. 5, II). As in foetus I, there occurs here also a 'jog' in the ninth band, which is approximately the 'mirror image' of that in the partner individual, in that there are 34 scutes on the right of the break and 30 on the left.

Foetuses III and IV both show a completed splitting of the first band into two, thus producing ten bands; and they also lack the irregularity in the ninth band.

Attention is called to the following points:

1. All four members of the set show an extensive splitting of the first band. The splitting was predetermined in so far as the location within a certain band is concerned, but the extent or manner of its expression appear to have been beyond the limits of hereditary control.

2. There was evidently a fairly rigid hereditary control in the matter of the various limits of the incomplete splittings, as one may judge by the facts that the two sides of foetus I are exactly identical and that six scutes constitute the marginal unsplit region in every case where the splitting is incomplete. This would indicate a precision of hereditary control almost as remarkable as that noted in set 64.

3. There is a pairing of foetuses on the basis of the extent of the splitting, pair A (I and II) showing an incomplete splitting, and pair B (III and IV) a complete one. Pairing is again evident in the matter of the irregularity in the ninth band, pair A (I and II) showing it, pair B (III and IV) lacking it.

4. The 'three-to-one' proportion appears again in that three individuals are bilaterally symmetrical and one is unilateral in the expression of the splitting process.

*Set 118 (Mother normal).* This is the only set in our collection where only one member of a set shows a band 'abnormality.'

Foetuses I, II and IV are normal.

Foetus III shows a fusion of small extent between the first two bands, involving only three scutes at the left hand margin.

The extent of the 'abnormality' is so slight that it seems scarcely worth while to discuss the possible bearings of the condition. It would appear best to consider the case as one of incipient fusion, which may have arisen epigenetically in one of the four individuals. In the next generation it might have been inherited in a more pronounced form. There is, however, the alternative explanation which is developed in the discussion of the theoretical causes of pairing.

*Set 121 (Mother abnormal).* This is one of two observed cases of the direct inheritance of an atypical condition from the mother. In this case the mother had a right marginal fusion of the last two bands, involving 4 scutes.

Foetus I is normal.

Foetus II shows a 'jog' in the ninth band similar to that shown in fig. 23 and in set 101. There are 33 scutes on each side of the break and hence the latter is median.

Foetus III is normal.

Foetus IV shows a 'jog' in the ninth band just like that in foetus II except that there are 33 scutes on the right and 35 on the left.

The 'abnormality' in the mother is entirely different in character from that shown by the two foetuses, but in that it is unilateral and involves the ninth band in both mother and foetuses, there would seem to be ground for believing that there is some genetic connection between the peculiarities seen in the two generations. It is quite possible that the parental and filial conditions may have no hereditary connection, but there must nevertheless be some predetermining mechanism controlling the occurrence of the same peculiarity in two of the members of a set.

It should be noted that the two diagonally placed foetuses seem to be paired with respect to the band irregularity, and that the same individuals were noted as paired on the basis of scute counts. This condition strengthens the hypothesis that occasionally there may occur such a shifting of the blastomeres as to bring about a false pairing in the arrangement of the quadruplets without interfering with their inherited potentialities. For a more detailed discussion of this situation reference is made to one of our former papers on this subject.<sup>1</sup>

*Set 123.* Mother shows a deep crease in the scapular shield, between the ninth and tenth scapular rows (counting from the posterior margin of the shield). This crease is apparently due to the suppression of part of a row of scutes.

All four foetuses show the same peculiar crease in precisely the same region. The photograph (fig. 24) will serve to emphasize

<sup>1</sup> Newman and Patterson, 1910. *This Journal*, vol. 21, no. 3, p. 401.

the striking identity of the four quadruplets with respect to the peculiarity on question. This is the only undoubted case of the direct transmission of a definite scute peculiarity from the mother to the offspring which we have encountered. In this case it seems clear that hereditary control and hereditary transmission are equally precise, and it appears probable that most of the cases we have described are inherited either from the unknown fathers or from some recent ancestor.

PAIRING; AN INTRA-FRATERNAL CORRELATION; AND ITS BEARING  
ON THE PROBLEMS OF HEREDITARY CONTROL

The most difficult question that confronts us in attempting to gain an insight into the operation of the predetermining mechanism concerns not so much the resemblances between the quadruplets as their differences. Why are they not all exactly alike and why are there closer resemblances between some individuals of a set than between others?

It has been shown that, with respect to their uterine connections, the four foetuses are definitely paired, in that two of them are attached to each of the lateral placental discs of the chorionic vesicle. Embryological investigations have revealed indications of a much earlier and more intimate pairing. With very few exceptions the resemblances between the foetuses are strictly in accord with their placental pairing, the closest resemblances occurring between the two individuals attached to the same placental disc. This is not a mere matter of arbitrary judgment, but is based on a comparison of the inter-pair and intra-pair correlation. By the use of the difference method previously employed the two constants have been determined as follows:

Inter-pair coefficient of correlation = 0.9257

Intra-pair coefficient of correlation = 0.9517

It will be noted that the correlation between the individuals of opposite pairs (inter-pair correlation) is lower than that determined for the whole set (.9348), while the correlation between the individuals of the same pairs is decidedly higher; in fact this is probably the highest correlation constant determined for any

organic relation. How surprisingly alike are the members of the pair may be realized by an examination of table 6. A glance down the column of totals will reveal the frequency with which exact identity exists between the paired individuals and how rarely such is the case between individuals of opposite pairs.

It might be claimed that any method of pairing would give a closer correlation than that derived from a treatment of the whole set. That this claim is without justification is readily shown by the experiment of pairing the fetuses in different ways and determining their correlation constants. There are two other possible ways of pairing the quadruplets. We may put together the adjacent members of opposite pairs, associating foetus I with IV and II with III, or we may pair them diagonally, associating I with III and II with IV. The result of the experiment is shown below:

Correlation coefficient, true pairs (I-II) and (III-IV) = 0.9517

Correlation coefficient, false pairs (I-IV) and (II-III) = 0.9270

Correlation coefficient, false pairs (I-III) and (II-IV) = 0.9394

It will be noted that the correlation constants for both of the false pairs differ only to a slight extent from that of the whole set; which shows conclusively that there is no intra-fraternal correlation on the basis of either of these artificial groupings.

The pairing relation is brought out even more strongly in the study of atypical scute and band conditions. In each set dealt with reference has been made to the instances of pairing as they occurred, and in several cases it was shown that pairing was exhibited in several different ways in the same set. No more convincing evidence of the reality of pairing could be asked for than is afforded by the conditions seen in sets 64, 96 and 101.

The fact of pairing would seem then to need no further demonstration; but its underlying causes and its relation to the mechanics of hereditary control are problems that require much consideration.

It must be frankly admitted that so far we have failed to prove conclusively that each foetus in a set is the product of a single blastomere of the four-cell stage. The youngest embryonic vesicles appear to be still single individuals so far as any visible

demarkation of embryonic primordia is concerned; but the absence of a visible line of separation between the quadrants of the vesicle does not preclude the possibility that each quadrant may be formed exclusively, or nearly so, of cells derived from one of the first four blastomeres. It seems very likely, in fact, that the cleavage products of each blastomere would continue to occupy the relative position held originally by the parent cell, in spite of the various complex developmental processes that ensue, just as the cells derived from the first two blastomeres, in many organisms, retain their bilateral positions and go to form the right and left sides of the individual. To this extent then we are probably justified in tracing back each of the quadruplets to one of the first four blastomeres. If this be granted it results logically that each pair must be the product of one of the first two blastomeres.

On this basis alone are we able to offer any reasonable explanation of the observed phenomenon of pairing or to attempt an answer to the question: Why should there be a closer resemblance between paired than non-paired individuals? Our original explanation of the condition naïvely assumed that the first cleavage would divide the fertilized egg into two somewhat unequal parts, and that the second cleavage would divide these half eggs into quarters more nearly equal than were the halves. In brief we assumed that the first cleavage gave products more variable than the second. Is there any basis for such an assumption? Is there, as development proceeds, a progressive decrease in the variability, real or potential, of daughter cells? It has been discovered from a study of the intra-individual variability of certain plants, notably *Ceratophyllum*, (Pearl, '10), in which whorls of leaves are successively produced by the apical bud or growing point, that the leaves of the first whorl are the most variable, least closely correlated, and that later ones vary less in an orderly progression. Similarly, if we consider that in the first cleavage the armadillo ovum divides itself into two potential individuals and that each of these in turn divides into two more individuals, we would expect to find a decreasing variability among the like parts produced at each successive division. In this case, however, the production of quadruplets destroys the twins and we can dis-

cover the variability of the products of the first division only by determining the inter-pair correlation in our sets of quadruplets. This constant should be lower than that determined between the individuals of the several pairs, the intra-pair correlation. This is exactly what we have done, and the results, as given, are in accord with the hypothesis. An interesting test of the validity of this explanation of pairing might be made in connection with the foetuses of *Tatu hybridum*, one of the South American armadillos, which has most commonly eight or more polyembryonic foetuses. Here the production of individuals has gone at least one step further than in our species, and it should be possible to find out whether the products of the last division are more closely correlated than are the paired individuals in our species or than the products of the previous division. It is to be hoped that some investigator to whom the material is available may see fit to satisfy our curiosity on this point. If our hypothesis should prove to be well founded we shall have furnished an interesting illustration of the law of decreasing variability with the production of like parts, which is probably a corollary of the more general law that variability decreases progressively with advancing development, a law made clear by Vernon in his book on "Variation in animals and plants."

What are the logical consequences of accepting such an explanation of pairing, and what light may the ideas expressed throw on the problem of the mechanics of hereditary control? Two courses are apparently open. We may consider the fertilized armadillo egg as heterogeneous in structure, so that its four quadrants, which occupy the positions of the four blastomeres, bear the different materials which determine the differences between the four foetuses. On this basis it would be difficult to explain the paired relation, and still more difficult to accept the necessary consequences of the assumption that, where certain atypical conditions occur in all four foetuses, the physical basis of the 'abnormality' must have been repeated four times over in as many quadrants of the egg. It would involve too severe a strain on one's credulity to ask him to believe that in sets 121 and 123, for

instance, the double scute primordium occurred separately in three of the quadrants of the uncleaved oosperm.

The alternative hypothesis involves the assumption that the differences are due to the lack of complete accuracy in the bilateral distribution of the hereditary materials (probably chromosomes). During the process of cleavage by means of the mechanism of hereditary transmission, whose visible operations are probably intimately associated with the mitotic figure, the various materials which condition the development of the definitive structures are distributed more or less unequally to the two daughter cells. The next cleavage involves another unequal distribution of materials, but the inequality is lessened; and presumably, as the cleavage process continues, either the distributing mechanism becomes more exact through practice or else the material distributed becomes progressively more homogeneous, and hence less apt to produce variability.

The Mendelian-like ratios which we have noted so often may be no more than fancied parallelisms, but we have been unable to dismiss the phenomenon without some speculation as to its possible significance. The true Mendelian ratio of 'three-to-one' is the result of the segregation of grand-parental characters, a fact which has suggested the thought that we may have here a condition involving in some way, which we are at present unable to understand, the interaction of dominant and recessive ancestral characters. The only alternative explanation of the presence of a character in three individuals of a set and its absence in the fourth involves the assumption of latency, whose aid we hesitate to invoke, because we fear that its generous assistance has already been presumed upon over much. After all there is still much to explain in connection with the problem in hand and we can scarcely expect to be able to solve some of its mysteries without the aid of breeding experiments. In spite of the many difficulties that confront us in connection with attempts to keep these animals alive in confinement and to have them breed under experimental control, we feel that breeding is imperative and that success will come in time.

## THE HEREDITARY CONTROL OF SEX

The only character that seems to be rigidly controlled—which does not vary at all in the members of a given set of foetuses—is the character of sex. Whether the set shall be male or female is apparently settled at the time of fertilization and has its physical basis presumably in a dimorphism of the spermatozoa. Sex having been determined, there is no room for individual variation with regard to this character, unless there be such a thing as a more or less pronounced maleness or femaleness. If degrees of sex do exist they no doubt express themselves in terms of fertility.

Along with the primary character of sex are predetermined all of the secondary sexual characters, but these are not so rigidly controlled. Probably no more highly variable characters exist than those that are associated with sex. In the armadillo the two sexes are remarkably alike with regard to somatic characters. So similar are the two sexes that we have never been able to tell them apart without examining the genitalia. A statistical study of the scutes has, however, revealed a sexual dimorphism in the scute numbers. The mean number of scutes in males is a little higher than that in females, but the difference is one of only four or five scutes in the whole banded region. A more pronounced dimorphism exists in the comparative variability of the two sexes. Not only in the species, but within the confines of the various fraternities, the males are decidedly more variable than the females. Is there any structural dimorphism of the hereditary materials that could be held to be in any way correlated with this variational dimorphism?

A preliminary study of the spermatogenesis of this species has revealed that a dimorphism of the spermatozoa probably exists. If the results of a more extensive study support this tentative conclusion, it will be possible to bring the question of the production of male and female sets into line with the general conclusions already reached on sex-determination in the other forms in which a dimorphism of the sperms brings about a balanced chromosome complex in the female and an unbalanced one in the male. Can there be any underlying connection between the bal-

anced chromosomal relations of the female and variational stability on the one hand, and between the unbalanced condition of the chromosomes in the male and its variational instability on the other? If such a connection exist we have, in addition to sex, another character whose physical basis may in some way be associated with the dimorphism in the chromatin content of the fertilized egg.

A further suggestion might be made in this connection. It seems to be an established fact that the male sex is the more highly specialized, for males depart more widely from the juvenile type than do females. Might it not be possible that the unstable equilibrium of the male chromatin complex lies at the basis of the higher specialization of the male; for a condition of instability would involve an increased potentiality for progressive change? Is there any more or less justification for these suggestions than there is for the generally accepted idea that sex is in some way causally associated with the presence or absence of the accessory chromosome, or its equivalent?

#### GENERAL CONSIDERATIONS

In his chapter on blastogenic variation Vernon quotes from Weismann the statement that "the individual is determined at the time of fertilization, or, in other words, the individuality of the organism results from the fact that the germ-plasm is composed of the paternal and maternal ids which are brought together in the egg cell." As evidence of the validity of this statement the author brings up the facts about human identical twins and on the basis of these facts claims that "heredity is potentially decided at the time of fertilization."

In the present paper we have shown that the individuality of the organism is not precisely determined at the time of fertilization, but that the characters are hereditarily controlled only within certain limits. These limits we have been able to define with respect to a number of different characters.

Our results are based on the assumption that the degree of divergence shown between the four foetuses is the index of the

variational potential of the fertilized egg. We assume that if the egg which produces a given set of quadruplets could be made to produce just one individual, this individual would have a potential range of variability equal to that exhibited by the set of quadruplets. Whether or not this assumption is justified depends to some extent on whether our ideas about the operations of the mechanism of hereditary control are sound. If one attribute the differences between the foetuses to the fact of the unequal distribution of the hereditary materials, he would seem to be forced to the conviction that, were a single foetus to develop, there would be no chance for the operation of such a factor, and hence the heredity would be settled at the time of fertilization. This conclusion does not appear to be so necessary when we consider that, even where the egg produces only one individual, it must still divide into two, four, etc., blastomeres, and that each division affords an opportunity for an unequal distribution of hereditary materials. In the first cleavage, instead of producing two distinct individuals, the egg divides its material into two bilateral halves, which are destined to produce the right and left sides of the definitive body. Now it has been shown in another connection that the correlation between the antimetrichally paired organs of the same individual is of the same grade as that shown to exist between the quadruplets. This fact simply confirms the idea that the variability of the sets of foetuses gives a reliable measure of the variational possibilities of the fertilized egg. Specific polyembryony doubtless furnishes a special case of intra-individual variability, in which the original individual breaks up into several strictly homologous and independent parts.

We have realized from the beginning of our work on this subject that the results obtained might appear to some biologists to be explicable on the basis of similar or different environmental influences operating during gestation. For these reasons we have been careful to select for study structures little if at all likely to be influenced by environmental factors. It will doubtless be claimed by some that the foetuses are almost identical because they have developed under almost identical conditions, and that the slight differences are the result of equally slight differences in

position, nutrition, etc. That the environmental factor cannot be seriously considered is shown when particular cases are examined. What kind of an environmental stimulus, for example, could operate to produce such a definitely localized, minute peculiarity as the double scutes in the individuals of sets 121 and 123? One could hardly imagine that a slight difference in the amount or character of the maternal nutriment would produce such a condition, nor could any mechanical factor, such as position, pressure or contact with the amnion, be held accountable for so definitely localized a character occurring in several individuals.

Again, in the matter of atypical band arrangements, it would appear equally absurd to attribute resemblances or differences to environmental factors. Consider, for example, the conditions in set 64. Here the region of incomplete splitting is so definitely localized and involves so fixed a number of scutes that one would have to posit some kind of environmental influence which would be able to cover just so many scutes and place itself just so far from the margin in every case. It would also be necessary to explain why two of the four individuals should show the effects of the influence bilaterally and why the other two should show it unilaterally.

These and many other cases that might be examined point to the untenability of the position taken by the proponents of the efficacy of environmental factors, and strongly fortify the position here taken, that the characters dealt with are purely of blastogenic origin. We conclude then that we have without question made an advance in the direction of determining the limits of hereditary control. We have shown with what degree of exactness the numbers of certain integral variates, the scutes, may be predetermined and how much room is allowed for the play of epigenetic factors. We have demonstrated that the alignment of scutes into bands is very largely controlled by mechanical factors. We have indicated the degree of exactness with which certain 'abnormalities' may be hereditarily controlled, and how small a biological unit is capable of predetermination.

One cannot but be impressed, however, with the diversity of conditions seen in the different sets. In some of the normal sets

it appears that hereditary control is almost perfect, as, for example, in set 4, where there is a difference of only one scute among the four foetuses; in other sets, 97 for example, the variation among the foetuses is so great that, were they not found to be enclosed in a common chorion, one would hardly believe them to be related. The same conditions prevail in connection with the atypical scutes or bands. In some sets the 'abnormality' is repeated in the different individuals with the utmost fidelity of detail, while in others neither the extent of the region affected nor its location is at all rigidly defined. In a word, one can speak definitely as to the limits of hereditary control for only one set at a time. What appears to be true for one case does not apply exactly to another.

Even here, then, where one would expect the phenomena of variation and heredity to exhibit almost diagrammatic simplicity, we find a high degree of complexity and lack of uniformity; and one is again compelled to acknowledge that nature is baffling in her manifoldness of expression and in her freedom from the trammels of exact laws.

#### SUMMARY

1. The data derived from human identical twins cannot serve as a criterion for the determination of the limits of hereditary control, for two reasons: (a) The origin of the two individuals from a single fertilized egg is assumed from the fact of resemblance. (b) The comparison between the twins is made only after years of post-natal life.

2. Armadillo quadruplets furnish a reliable substitute for human identical twins, because: (a) The phenomenon of specific polyembryony has been demonstrated for the species. (b) The unborn foetuses, with all placental connections intact, are used. (c) The scutes of the nine bands of armor, which are the objects of the present investigation, are elements that reach their definitive number and arrangement long before birth, and hence are excellent for the study of heredity.

3. A study of the morphology of the integument reveals the fact that the integumentary unit is a complex element made up of a bony plate, a horny scute and a well defined hair group.

These are so closely associated and so definitely inter-related that a study of one element furnishes an index of the variability of all. For convenience, the external element, the scute, is chosen for statistical study. When the term 'scute' is used the whole complex is to be understood.

4. A statistical study of the species variation in the nine bands reveals the facts that we are dealing with a highly variable character which fluctuates according to the laws of chance. The mean, mode and median practically coincide, and the observed and the theoretical variation curves are very similar.

5. Males are decidedly more variable than females with respect to the characters studied.

6. The variability of the bands taken separately is proportionately greater than that of the banded region as a whole. In each band, however, the variation in the numbers of scutes appears to take place according to the laws of chance.

7. The twenty sets of normal fetuses furnish an ideal array for the study of fraternal correlation. Taking each set as a fraternity, and dealing with the total number of scutes in the banded region, a correlation coefficient of .9348 is obtained. This is taken as an index of the strength of hereditary control with respect to the character in question. The only correlation constants at all comparable with this are those derived from a study of the anti-metrically paired organs of the same individual. This fact confirms the idea of the polyembryonic origin of the quadruplets, and shows that, morphologically, we are dealing with four parts of one individual.

8. The correlation coefficients determined for the individual bands are comparatively so low that the conclusion is reached that the process of scute alignment is largely mechanically determined and hence beyond the limits of hereditary control.

9. A study of the atypical variation in the banded region shows that there are several types of scute 'abnormality,' double scutes, split scutes and the three-hair type of scutes; and also several types of band 'abnormality,' fusions, splittings and additions. All of these conditions are comparatively rare and highly diversified in detailed expression.

10. Practically all of the types of atypical variation are found in the present collection of foetuses. They are shown to be predetermined, in some cases, with remarkable precision, and in other cases, only in so far as their general character and location are concerned.

11. A further statistical study of pairing serves to demonstrate the truth of this relation. A mechanistic interpretation of pairing is offered, involving the idea that the differences in the four foetuses of a set may be due to the inexactness of the distributing mechanism in cleavage. The paired condition is thought to be an illustration of the general law that variability decreases with the production of like parts.

12. Sex is the only character absolutely predetermined, but the suggestion is made that the greater variability of males, both in the species and within the several sets, may be associated with the lack of balance in the chromosome complex.

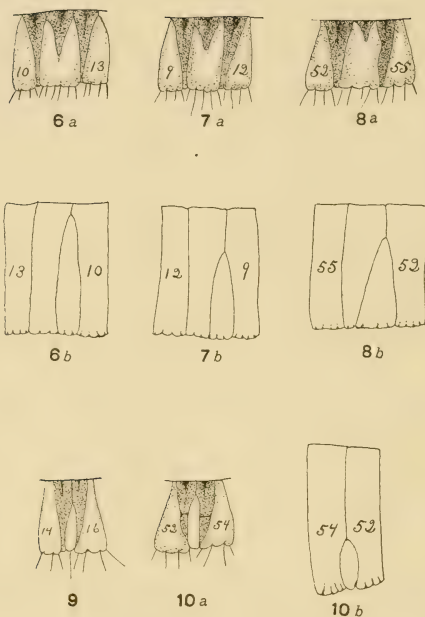
13. In reply to the possible objection that the variability of the four foetuses of a set is not necessarily an index of the possible range of variability of the ovum, it is argued that the variation between the right and left sides of the body of a single individual is of the same grade as that shown to exist among the quadruplets, and hence, from the variational standpoint, the single foetus is on the same footing as the quadruplets.

14. The conditions described are shown to be inexplicable on the basis of varying environmental factors acting during gestation.

15. The lack of uniformity in the different sets of foetuses, with respect to the limits of hereditary control, leads to the realization of the complexity of the problem and to an acknowledgment that we are still far from a complete understanding of the factors involved.

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Figs. 6-8 These figures show the upper and lower surfaces of three double plates in various degrees of fusion.  $\times \frac{3}{4}$ .

Figs. 9 and 10 Two specimens showing incomplete plates.  $\times \frac{3}{4}$ .

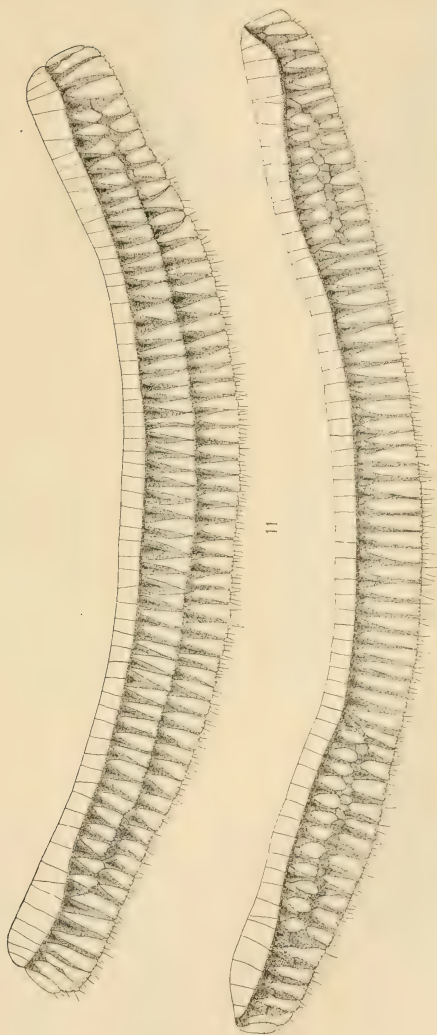
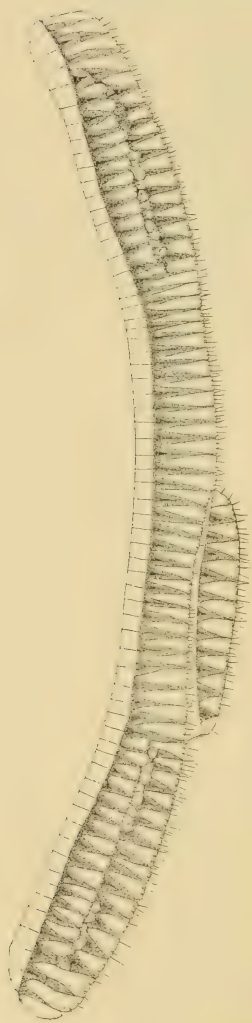
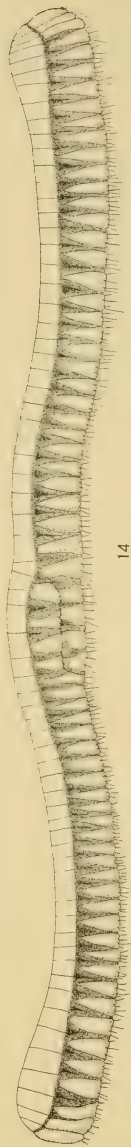


Fig. 11 A specimen with a bilateral type of fusion between the first and second bands.  $\times \frac{1}{2}$ .  
Fig. 12 The first band of a specimen showing a bilateral splitting.  $\times \frac{1}{2}$ .



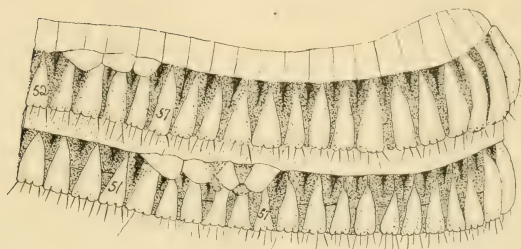
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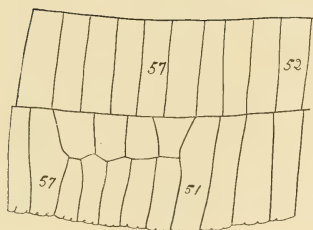
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Fig. 13 Similar to the preceding, except that there is an additional split on the left which has resulted in producing a short incomplete band.  $\times \frac{1}{2}$ .

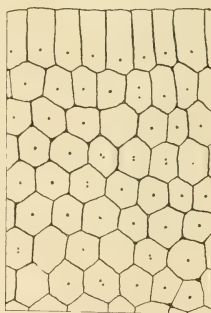
Fig. 14 The ninth band with a short double region lying approximately at the middle point of the band. This may have been produced by an imperfect meeting of the right and left primordia during the process of conecrescence.  $\times \frac{1}{2}$ .



15 a



15 b



16

Fig. 15a Right-hand ends of the 8th and 9th bands of a shell showing two slight 'abnormalities,' for a description of which see text.  $\times \frac{3}{4}$ .

Fig. 15b Under surface of the affected regions of the preceding.  $\times \frac{3}{4}$ .

Fig. 16 Under surface of a portion of the anterior half of the pelvic shield. Note the hexagonal shape of a majority of the bony plates. These are all perforated by one or two small holes through which blood vessels and nerves pass.  $\times \frac{3}{4}$ .



Fig. 17 Snap shot of a living animal.  $\times \frac{1}{6}$ .



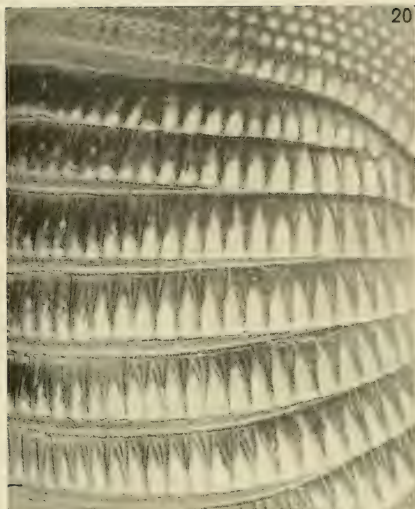
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Fig. 18 A shell showing a fusion between bands 1 and 2.  $\times \frac{1}{5}$ .

Fig. 19 A shell showing an addition to the banded region from the scapular shield.  $\times \frac{1}{5}$ .



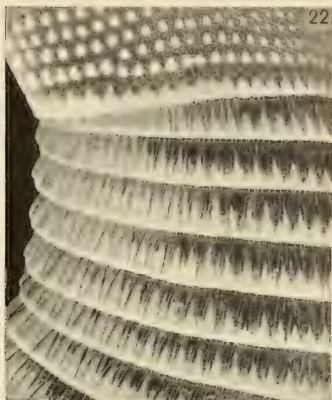
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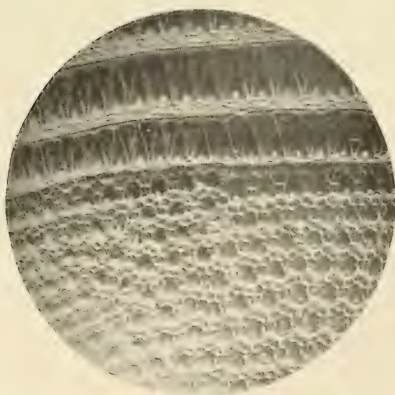
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Fig. 20 Photograph of a portion of the left side of a shell. This shows the condition of the primary and secondary scutes.  $\times \frac{2}{3}$ .

Fig. 21 Photograph of the upper surface of the pelvic shield (also part of the banded region), to show the pebbled effect.  $\times \frac{1}{3}$ .



22



23

Fig. 22 Left side of the shell with the high count of scutes (625), many of which are of the 3-hair type. A number of these can be made out in the photograph, especially in the first and second bands.  $\times \frac{1}{2}$ .

Fig. 23 The middle portion of the pelvic shield of a specimen showing a 'jog' in the region of the ninth band.  $\times \frac{2}{3}$ .



Fig. 24 Dorsal view of the anterior ends of the litter of embryos from female no. 123. The embryos all show a well marked crease in the middle of the scapular shield. The mother also had this same peculiarity. Note the double scute in the middle of the last row of the scapular shield of each of the two embryos lying on the right. These two embryos are members of one pair.  $\times \frac{3}{4}$ .

# EXPERIMENTS ON THE CONTROL OF ASYMMETRY IN THE DEVELOPMENT OF THE SERPULID, HYDROIDES DIANTHUS<sup>1</sup>

CHARLES ZELENY

SEVEN FIGURES

## INTRODUCTION

The following experiments<sup>2</sup> were made as part of a study of the factors controlling asymmetry in the Serpulid, *Hydroides dianthus*. The operations were performed on young individuals in which asymmetry was just starting to develop and in which the adult mechanism for reversal of the opercula was not yet present. The results are interesting not only in connection with the problem of the cause of the original asymmetry and of the reversal of the opercula in adults but also as bearing on the extent of agreement between regeneratory and ontogenetic stages. A more definite formulation of the problems follows.

1. The first functional operculum of young animals is developed before the first rudimentary operculum. In the absence of any special mechanism in the form of a rudimentary operculum does reversal of position follow removal of the functional organ at this stage? What bearing does the behavior following such operations have on the question of the origin of the original asymmetry and the cause of reversal of the opercula in adults?

2. The first functional operculum of the young is different in type from that of the adult. Does the regenerated operculum

<sup>1</sup> Contributions from the Zoological Laboratory of the University of Illinois, No. 8.

<sup>2</sup> The experiments were performed at the Biological Laboratory of the Bureau of Fisheries at Woods Hole, Mass., during July and August, 1909. I am indebted to Dr. Francis B. Sumner, the director, and to Professor Raymond C. Osburn, acting director during a part of my stay, for many courtesies. A preliminary report of the experiments was given before the Central Branch of the American Society of Zoologists at the Iowa City meeting, April 8, 1910.

resemble the one removed or does it develop directly as an operculum of the adult type?

3. Further, since the first operculum is a modification of a branchia and therefore originally has a respiratory function, is its removal followed by regeneration, first as a branchia which only later develops the opercular modification, or is the opercular modification regenerated directly?

For a description of the opercula in adults and for experiments on reversal, reference is made to two former papers (Zeleny '02, '05). The development of the opercula in the young is treated in the second of these (pp. 38 to 54). A brief outline of the necessary points must suffice here.

#### ASYMMETRY IN ADULTS

In the adult *Hydroides dianthus* there is a large functional operculum or tube plug on one side of the body, either right or left, and a small rudimentary operculum on the other side (fig. 1). The functional operculum (fig. 1, *F*) has a stout stalk ending in a hard chitinous enlargement consisting of a serrated cup, from the centre of which rises a circle of curved spine-like projections. The genus *Hydroides* is characterized by the presence of these two separate circles of serrations or projections in its functional operculum. The rudimentary operculum (fig. 1, *E*) is a small bud-like structure corresponding in position exactly with the functional operculum of the opposite side of the body. Near the base of each opercular stalk there is a well defined line, the breaking line or breaking joint.

When the cup of the functional operculum is removed, the rudimentary operculum of the opposite side develops into a functional operculum similar to the one which had been injured. The stalk of the injured operculum meanwhile drops off at its breaking joint and a rudimentary operculum develops in its place. There is thus, as a final result of the operation, a reversal in position of the two opercula. This reversal may be repeated several times.

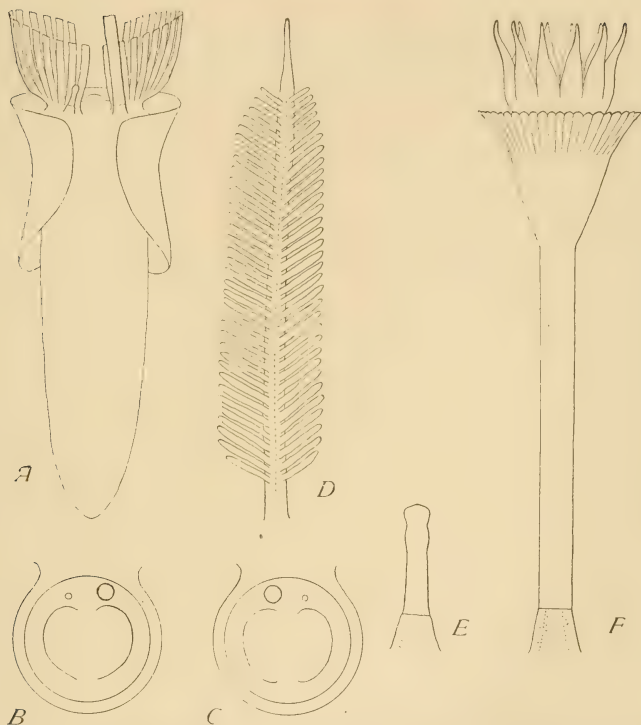


Fig. 1 *Hydroides dianthus*. *A*, dorsal view of right-handed specimen, showing relations of parts. Ends of branchiae and functional operculum not given ( $\times 6$ ); *B, C*, diagrams of anterior surface of head of left-handed and right-handed specimens ( $\times 6$ ); *D*, branchia viewed from inner surface ( $\times 25$ ); *E*, rudimentary operculum ( $\times 30$ ). *F*, functional operculum ( $\times 30$ ).

The process is very similar to the reversal of the two chelae in *Alpheus* as described by Przibram ('01 to '07), Wilson ('03), Zeleny ('05) and Stockard ('10). In *Alpheus* there is a larger so-called 'snapping' chela on one side of the body and a smaller 'cutting' chela on the other side. These chelae differ from each other both in size and in structure. When the snapping chela is

removed, the cutting chela changes into a snapping chela and in place of the former snapping chela a cutting one is developed.

In *Hydroides* the presence of the functional operculum in some way inhibits the growth of the rudimentary operculum into a functional one. Likewise in *Alpheus* the presence of the snapping chela inhibits the change of the cutting chela into a snapping chela. It is evident that the rudimentary operculum in the one case and the cutting chela in the other need only the proper stimulus or removal of an inhibition to develop at once into organs like their mates. These smaller organs are therefore in one sense merely stages in the development of the others.

Various suggestions as to the explanation of this inhibition have been made. In *Hydroides* the injury to the large operculum produces activity on both sides of the body, bringing about the immediate growth of the rudimentary into a functional operculum and a start in the same direction to form a rudimentary operculum on the side of the injury. The functional operculum is reached only on the side with the earlier start, the presence of the one functional operculum restraining the possible development of another functional one. This view is supported by the result obtained when the head of *Hydroides* is removed. In this case, as also in a part of the cases in which the two opercula are removed without injury to the body proper, a functional operculum is developed on each side of the head though the size of each is usually reduced. In such a case the two opercula get an equal start and neither one is able fully to restrict the development of the other.

The cases of similar chelae in the adult *Alpheus* and *Homarus* may be explained in a similar way by coincident development. Under the ordinary conditions of removal of both chelae the advantage of greater blood-supply and probably other features lies on the side of the stouter snapping or crushing chela. Therefore reversal does not occur in such cases. When, however, there is a secondary advantage of just the proper strength occurring to the side of the former smaller chela two equal chelae may develop. The probability of this explanation is strengthened by the fact that in those cases in which two equal snapping or crushing chelae

were obtained by Wilson ('03) and Emmel ('08) there was an additional factor, difference in time of removal of the two chelae, injury of the nerves of the chelae or the removal of the walking leg or legs on the side of the former more slender chela (see Stockard '10, discussed below).

Wilson has suggested that the reversal may be under nervous control and he made a study of it in *Alpheus* by cutting the nerves going to the chelae. His results, as he himself recognized, are, however, not conclusive, since other influences, such as disturbed blood-supply, are not eliminated. Wilson also suggests that the snapping chela may develop always on the side of the body which has the greater amount of material to start with and which therefore has the greater body of nutrition directed to it. Stockard has tested this hypothesis as applied to the whole group of appendages on the two sides, by removing the walking appendages on the side of the cutting chela in the case in which the snapping chela is removed. The operation leaves the greater mass of appendage material on the side of the chela removed. Nevertheless, practically all of the *Alphei* showed reversal as in cases without removal of walking appendages. Variations of these experiments showed the same negative result. The removal or non-removal of other appendages on either side has no proved relation to the phenomena of reversal. It should however be said that Stockard's experiments do not test the essential point of the hypothesis of difference in amount of material or effective mechanism for growth in the form of blood-supply, etc., between the stumps of the two chelae themselves. The walking legs may not be directly concerned in the difference of activities of the two sides at the first chela level and the hypothesis of difference of materials at the first chela level be unaffected thereby. Furthermore, if there be a correlation between these different levels, is it proper to assume, as Stockard has done, that the smaller amount of nutritive activity will be on the side regenerating other appendages. As a matter of fact, the opposite assumption is indicated by some experiments (Zeleny '09), and Stockard's experiments themselves, as far as they show any difference in results between the two cases, point in the same direction (Stockard '10).

## ASYMMETRY IN YOUNG INDIVIDUALS

The adult asymmetry of Hydroides is preceded by a symmetrical stage. With the development of a functional operculum the first asymmetrical phase is assumed, followed in turn by a normal reversal in position and a change in structure to the adult type. One or more additional reversals may then take place. It was shown that these reversals occur in nature and furthermore it is probable that they may come periodically without being preceded by any definite injury to the functional operculum other than the wear of ordinary use.

The free-swimming larva of Hydroides, after attachment to a solid object, secretes a tube around its body and develops branchiae on its head. There is a definite stage in which the branchiae are all alike and symmetrically arranged with respect to the median line (fig. 2). Additions to these branchiae take place at the lower edge of each lateral group. At a stage slightly older than the one shown in fig. 2 the young serpulid closely resembles in its branchial characters an adult of the genus *Protula* (Fritz Müller '64). There is no trace of an opercular modification in any of the branchiae.

The branchia next to the dorsal one on the left side then begins to form a terminal cup with a single row of serrations (figs. 3 and 4). The branchial filaments are still present and the respiratory function is evidently still retained along with the new tube-plug function. The opercular branchia at this stage may be compared with the functional operculum of adults of the genus *Apomatus*, in which also the opercular enlargement is on a stalk bearing branchial filaments though the enlargement itself is different in structure. *Apomatus* has in addition a rudimentary operculum of the same type as the functional, *i.e.*, borne on the end of a branchia which retains its respiratory filaments (fig. 6, *A, B, C, D,*). It is important to note that the operculum in *Hydroides* first appears on the left side of the body while in adults it is sometimes on the right and sometimes on the left.

During the following period the branchial filaments disappear from the opercular stalk and at the same time the corresponding

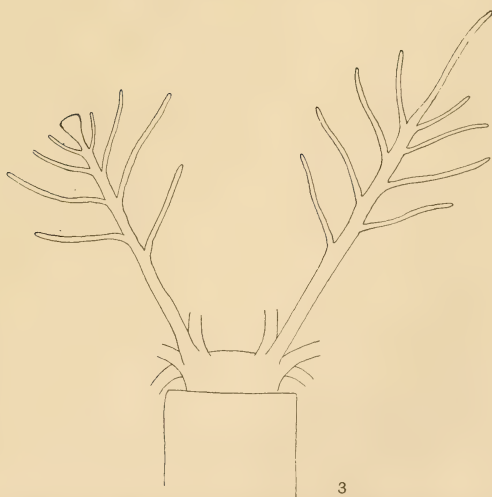
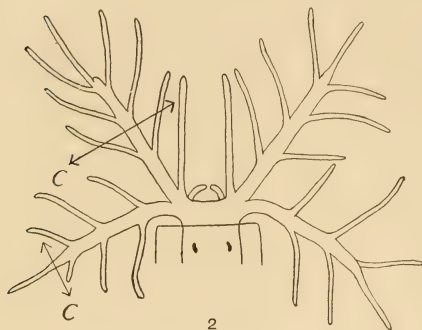


Fig. 2 Young *Hydroides dianthus* with six branchiae (individual *C*, no. 2780). The most dorsal pair has not yet developed its pinnules. The levels of the cuts made in the operation are indicated by *C*.

Fig. 3 *Hydroides dianthus*, age 34 days; stage with four pairs of branchiae. The next to the dorsal one on each side is shown in full. The left one shows the beginning of the knob of the functional operculum. The right one drops off later and the first rudimentary operculum is developed from its stump.

branchia of the opposite side drops off, the break appearing at the base of its stalk. In its place a bud-like rudimentary operculum is developed (fig. 5). There is then on the left side a functional operculum with a naked stalk and a terminal cup bearing

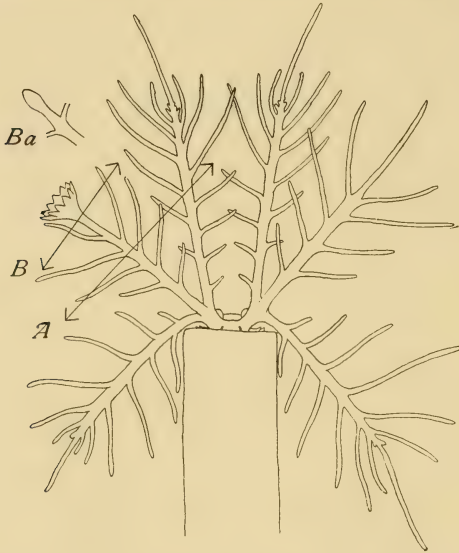


Fig. 4 *Hydroides dianthus*, age 26 to 28 days but further advanced than the individual shown in fig. 3. Stage with four pairs of branchiae. Figure shows the three most dorsal pairs. The ventral pair is directed away from the observer and is not shown. The opercular knob of the left side is notched and its stalk still retains the respiratory pinnules. The branchia next to the dorsal one of the right side has eight pinnules but differs from the other branchiae, except the opercular one, in the absence of new pinnule buds. *A*, level of cut in individual *A*, (no. 2778); *B*, level of cut in individual *B*, (no. 2779); *Ba*, regenerating functional operculum one day after operation in individual *B*.

a single row of serrations, and on the right side a rudimentary operculum. The characteristics are those found in the adults of the genus *Serpula* except for the fact that in *Serpula* the functional operculum may be either right or left (fig. 6, *E*).

After a period of activity in this stage lasting several days the functional operculum drops off. The loss is accompanied by the development of the rudimentary operculum into a functional operculum not like the one that had been lost but more complicated in structure, with two distinct and qualitatively different

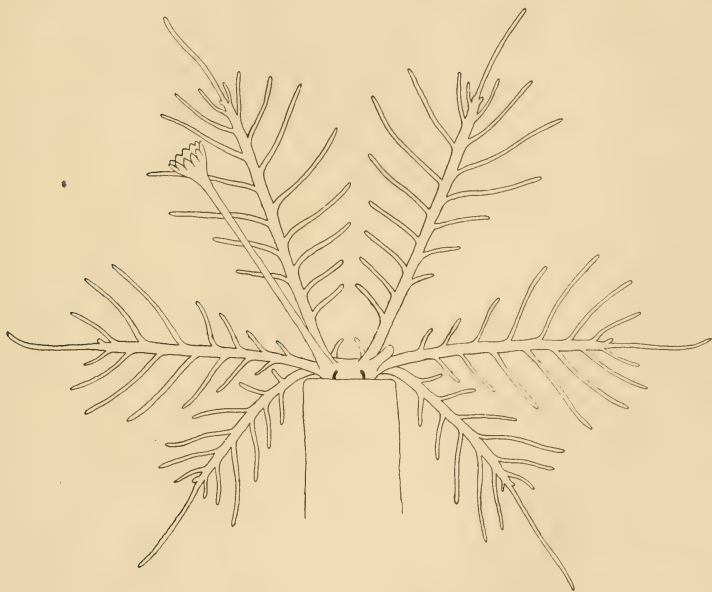


Fig. 5 *Hydroides dianthus*. Dorsal view ( $\times 38$ ). On the left side is functional operculum of *Serpula* type with naked stalk and one row of serrations. On right side is rudimentary bud developed from the base of the cast-off second branchia of that side.

rows of serrations. This operculum is similar to that of the adult *Hydroides*. In place of the operculum that had dropped off a rudimentary operculum is developed. The condition is now like that of the adult except that some adults have the functional operculum on the left and others on the right. It is therefore neces-

sary to assume further reversal or reversals of position during later development and perhaps throughout adult life.

The original asymmetry is apparently always of the same nature, *i.e.*, the functional operculum always develops on the left

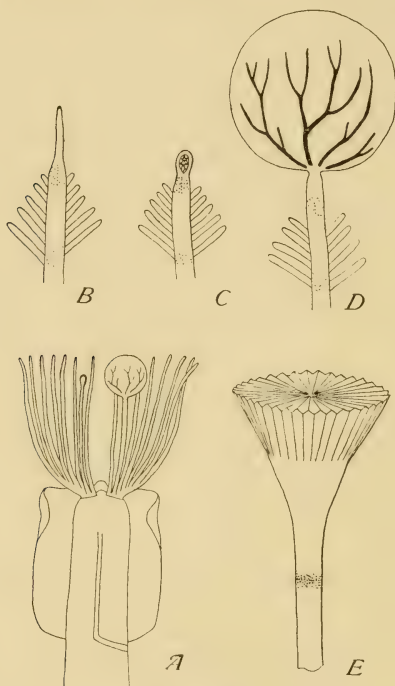


Fig. 6 A, B, C, D, *Apomatus ampullifera*. Adult. A, dorsal view showing functional and rudimentary opercula and branchiae. Pinnules not represented ( $\times 5$ ): B, tip of non-operculate branchia ( $\times 17$ ). C, tip of rudimentary operculum ( $\times 17$ ): D, tip of functional operculum ( $\times 17$ ): E, distal portion of functional operculum of *Serpula vermicularis* ( $\times 19$ ).

side and from a particular branchia. Adult individuals differ in character because some have had an odd and some an even number of reversals.

## EXPERIMENTS ON ASYMMETRY IN YOUNG INDIVIDUALS

The first functional operculum appears before the first rudimentary (fig. 4). During the stage in which the opercular stalk retains its branchial filaments there is as yet no modification of the branchia of the opposite side. It ends in a point just like that of other branchiae. Buds of new pinnules are however absent (figs. 3 and 4). There is at this time no perfected mechanism for reversal as in the adult. The removal of the functional operculum, or at an earlier stage the removal of the end of the branchia which later develops the opercular enlargement, was accomplished in several individuals (figs. 2 and 4). In only a few of them, however, was the operation clean cut and the further history of the experiment followed.

The character of the material did not allow experimenting with narcotization. It was therefore necessary to keep watch of the young animals under a binocular microscope, holding a needle knife blade above the opening of the tube. With exceptional good fortune it was possible to remove the terminal cup of the operculum in a few instances without seriously injuring the rest of the animal, though in most cases the neighboring branchia of the same side was also injured.

There was no reversal of opercula, though interesting developments followed. In place of the removed functional operculum a new one like the one removed was developed. There was no loss of the old stalk, the regeneration taking place directly at the cut surface (fig. 4, *Ba*). It might have been expected that, in case regeneration occurred, the new operculum would at once grow out as one of the *Hydroides* type as found after the first natural reversal. This, however, did not occur. The regenerated structure therefore repeats the stage of the removed structure and does not pass on to the next ontogenetic stage (fig. 7, *A*).

The effect upon the branchia of the opposite side is also interesting. The terminal part of the branchia develops a small knob, approaching in character a rudimentary operculum of this species but formed from a group of cells which in normal growth never develop opercular enlargements, but are, in fact, lost when

this branchia drops off to make place for the rudimentary operculum developing in its place (fig. 7, *B*, *C*). A branchia with a small knob-like rudimentary operculum at its end is thus formed. This condition is never found in normal development in this species but is a normal feature of the adults of the genus *Apomatus* which have also functional opercula with stalks bearing branchial filaments (fig. 6, *A*, *B*, *C*, *D*).

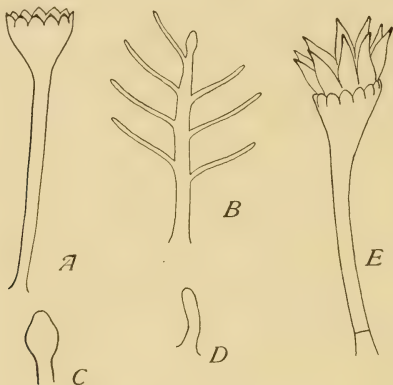


Fig. 7 *A*, regenerated functional operculum of *Serpula* type. Individual *A* (no. 2778), on August 3rd. *B*, branchia next to the dorsal one of right side as modified, following operation on the corresponding branchia of the left side. Individual *A*, (no. 2778) July 31. *C*, enlarged tip of *B*. *D*, rudimentary operculum on left side of individual *A* as developed after the functional operculum of the *Serpula* type had dropped off, August 14. *E*, functional operculum of *Hydroides* type as developed on the right side of individual *A* after the functional operculum of the *Serpula* type on the left side had dropped off, August 14.

It might have been expected that the removal of the functional cup would accelerate the breaking off of the corresponding branchia of the other side of the body and be followed by the development of a normal rudimentary operculum. As a matter of fact, the acceleration of opercular development is found, but in a way entirely different from the normal. A group of cells at

the tip of a branchia is stimulated to develop. This condition, however, lasts only a short time. The branchia with its terminal enlargement breaks off as does the corresponding one in normal development and a rudimentary operculum grows out in its place. While this is taking place the stalk of the regenerated functional operculum loses its filaments and the resultant condition is like that of the *Serpula* stage of normal ontogeny (fig. 5).

*Outline of typical individual experiments*

*Individual A. (No. 2778)*

*July 1—1909.* Egg fertilized.

*July 27.* Condition of opercula (fig. 4).

*Left side.* Second branchia has opercular enlargement. Respiratory pinnules still present.

*Right side.* No opercular modifications. Each branchia ends in a long tapering filament.

*Operation.* Distal half of second left (opercular) branchia and distal third of first left branchia removed (fig. 4, level A).

*July 31.*

*Left side.* Removed parts are regenerated. The new operculum resembles the removed one.

*Right side.* The next to the dorsal (second) branchia has developed a small ovoid enlargement at its end (fig. 7, B, C). The whole branchia now resembles a rudimentary operculum of the kind found in adults of the genus *Apomatus* (fig. 6, A, C).

*August 3.*

*Left side.* The new opercular stalk has lost its respiratory pinnules. The terminal cup retains a single circle of serrations (fig. 7, A). The structure resembles an adult operculum of the genus *Serpula* (fig. 5).

*Right side.* The next to the dorsal (second) branchia with its small terminal enlargement has dropped off and a rudimentary operculum has developed in its place (fig. 5).

*August 7.*

*Left side.* Functional operculum of *Serpula* type (fig. 5).

*Right side.* The former rudimentary operculum is going forward in its development to a functional operculum with two rows

of serrations (*i.e.*, of the Hydroides type). The new operculum is about half developed.

*August 14.*

*Left side.* The functional operculum of the Serpula type has dropped off and a rudimentary operculum has developed in its place (fig. 7, *D*).

*Right side.* The functional operculum of the Hydroides type is fully developed (fig. 7, *B*).

*Individual B. (No. 2779)*

*July 1.* Egg fertilized.

*July 28.* Condition of opercula as in no. 2778 on July 27.

*Operation.* The opercular enlargement at the end of the branchial operculum was removed (fig. 4, level *B*).

*July 29.*

*Left side.* Removed opercular cup is regenerating (fig. 4, *Ba*).  
*August 3.*

*Left side.* A functional operculum of the Serpula type is fully developed. The respiratory pinnules have already disappeared (fig. 5).

*Right side.* The next to the dorsal (second) branchia has dropped off and a rudimentary one has developed in its place (fig. 5).

*August 7.*

*Left side.* Functional operculum of Serpula type.

*Right side.* The rudimentary operculum has developed into a half-grown operculum of the Hydroides type.

*August 14.*

*Left side.* Rudimentary operculum (7, *D*).

*Right side.* Functional operculum of Hydroides type (7, *E*).

*Individual C. (No. 2780)*

*July 1.* Eggs fertilized.

*July 28.*

*Condition of opercula and branchiae.* Three pairs of branchiae are present. The most dorsal pair has not yet developed its pinnules. There is no sign of opercular enlargement (fig. 2).

*July 29.*

*Operation.* The distal half of the left second or future opercular branchia and the tip of the left third branchia were removed as shown in fig. 2.

*August 3.*

*Left side.* From the branchia next to the dorsal one a functional operculum of the *Serpula* type has developed. The stalk has already lost its pinnules (fig. 5).

*Right side.* Rudimentary operculum (fig. 5).

*August 7.*

*Left side.* Rudimentary operculum.

*Right side.* Functional operculum of *Hydroides* type, one-half developed.

*August 14.*

*Left side.* Rudimentary operculum (fig. 7, *D*).

*Right side.* Functional operculum of *Hydroides* type. Not fully developed as yet (fig. 7, *E*).

The experiments thus answer the three questions proposed at the beginning of the paper.

1. Is there a reversal of opercula as a final result of removal of the first functional operculum before any rudimentary opercular structure has been formed? The experiment shows that there is no reversal of opercula. A new functional operculum develops in place of the removed one, and on the opposite side of the body the final result is a rudimentary operculum like the normal one. Before this final condition there is, however, the interposition, as a result of the operation, of a new kind of opercular modification, namely, a rudimentary opercular enlargement at the end of a branchia. The enlargement is formed from cells which do not normally produce an operculum. The formation of the structure is directly stimulated by the operation. It has, however, no permanent result, the operculum thus formed in no way preventing the dropping off of the branchia.

2. Is the removal of the first functional operculum followed by the regeneration of a structure of the same type as the one removed or does the later or adult type develop at once? It is shown that the new structure is like the one removed. It does not skip to

the next succeeding stage. At this step, as probably at other steps in the ontogenetic process, removal is followed by a repetition of the part removed. The stages need not follow in a definite prescribed order. A stage may be repeated when the necessary stimulus is given. In normal ontogeny the first functional operculum has a period of existence as such which ends by its breaking off near the base. In its place a rudimentary operculum is developed, an operculum which is a preliminary stage in a new functional, the second of the Hydroides type, which develops only after a long period of latency. If, however, the first operculum be lost early in its life, there is no skipping of the later phases leading up to a normal loss, but the first stages are repeated and an operculum like the one removed results.

3. Is the removal of the first functional operculum followed by regeneration, first as a branchia which only later develops the opercular enlargement or is the opercular modification developed directly? The opercular modification is developed directly. An enlargement at the cut surface of the stalk is evident very soon after the operation. There is no trace of the development, first, of a pointed end like that of the original branchia, but opercular growth proceeds directly at the regenerating surface. There seems thus to be no repetition of the ontogenetic branchial stage at this regeneration (fig. 4, *Ba*).

The observations and experiments on young Hydroides contribute the following data on the factors controlling asymmetry of the animals.

1. The animal is originally symmetrical and the appearance of the asymmetrical structure, always on the left side, can not be due to the preponderance of nutrition on one side as a result of a larger amount of material on that side. There is further no indication that the original asymmetry is due to the character of the tubes or the nature of their curvature.

2. The removal of a functional operculum is not necessarily followed by the development of a rudimentary operculum on the same side. The larger mass of material, following the removal, was on the opposite side of the body. Nevertheless, the func-

tional operculum developed from the cut surface. A small opercular knob at the end of the branchia of the opposite side was, however, developed as a result of the operation.

3. The result of the operation was an animal with the same symmetry as a normal individual.

#### SUMMARY

1. The removal of the first operculum of *Hydroides dianthus* in its early stages before the development of a rudimentary operculum is followed:

A. By the regeneration of a new functional operculum of the same type as the one removed. There is no reversal of opercula such as occurs in the adult.

B. (1). The branchia occupying the position of the future rudimentary operculum and which in normal development shows no opercular modification develops an opercular enlargement at its end as a result of the operation.

(2). The opercular enlargement is developed from cells which in normal development do not form an operculum.

(3). The enlargement is of the rudimentary type such as is found in adults of the genus *Apomatus*.

2. The regenerated functional operculum is like the one removed. Regeneration does not go directly to the next succeeding or *Hydroides* type of the operculum. On the other hand neither is there a repetition of the preceding stage, the opercular enlargement appearing directly without the interposition of a branchial tip, later to be modified into an opercular structure.

3. The results point to the conclusion that reversal of opercula in the adult is dependent upon the presence of a specialized structure, the rudimentary operculum, capable of developing rapidly into a functional operculum. In the absence of such a special structure the regenerating tissue on the old functional operculum side retains its supremacy, getting an earlier start and inhibiting the development of a similar structure on the opposite side. The injury to the functional operculum does, however, initiate an

opercular modification in the branchia which stands in the position of the future rudimentary operculum. This process, however, is not sufficiently rapid to gain the upper hand and cause the development of a functional operculum.

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# ANATOMICAL ILLUSTRATION BEFORE VESALIUS<sup>1</sup>

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TWENTY-THREE FIGURES

The study of anatomical illustrations before Vesalius is not chiefly of antiquarian interest. It brings under consideration a momentous period of intellectual development when the scientific spirit was awakening and struggling for better expression. The examination of the human documents containing the early attempts at pictorial representation of the results of observation, have a peculiar interest for those who are still engaged in observing and recording results by the graphic method. Moreover, the consideration of these crude sketches reveals to us the conditions under which scientific men worked, the mental habit of the period, the educational practice in science and the degree to which accurate observation in anatomy prevailed. Nothing else shows more definitely the state of anatomical knowledge of the time, that which is covered and rendered ambiguous in the text stands exposed in the sketches—these graphic indices show the degree of fidelity to nature of the observer and his mental bias in the matter of interpretation.

<sup>1</sup> The notable interest of Professor Whitman in the historical phases of his science makes it appropriate that one of his students should offer in his memory a study of anatomical illustration before Vesalius—a study in the awakening of the scientific spirit.

Doctor Whitman was a pioneer in the United States in inaugurating university instruction in the history of comparative anatomy and of generation (see the Clark University Register for 1890). It is with pleasurable reminiscences that the writer acknowledges the influence of Dr. Whitman in the development of his mental interests. The friendly as well as the preceptorial relations with this leader of biological thought were a source of stimulus, especially as regards the philosophical outlook on nature, and the growth of a disposition to view current biological thought and attainment in the light of its historical development.

The pursuit of science from the historical standpoint has appealed only to a limited number, and there is needed at present a sympathetic recognition by scientific men, in general, that this affords a worthy field of research. This conception is being promoted by the relatively new movement in European universities, that has resulted in the appointment of professors of the history of medicine and natural science, to the establishment of periodicals devoted to researches in the same field and to the foundation in Leipzig of an Institut für Geschichte der Medizin. All this, and the growing disposition to provide a historical background for courses in biological study, is a sign that there is to be a widening of the field of biological research. It is to be hoped that the time is near when this line of study will be a recognized division of biological research, running parallel with other forms of biological investigation, and pursued as a research subject by examination of the original sources.

The attempts at pictorial representations of anatomy began before the invention of printing, as is shown in the pen, crayon and chalk drawings of anatomical subjects found in the medical manuscripts stored in the libraries at Berlin, Paris, Oxford, Munich and other places. A rich series of these manuscript anatomical sketches has been brought to light by Karl Sudhoff and his collaborators, and reproduced by photographic methods in the *Studien zur Geschichte der Medizin* and in the *Archiv für Geschichte der Medizin*. These resurrected manuscript sketches have thrown a flood of light on the sources of early anatomical illustrations. A genetic connection has been established between some of them and the earliest printed anatomical figures.

The question arises in connection with the early printed illustrations: Are these sketches crude representations of actual dissections, or are they based upon earlier traditional diagrams? They are in reality mixed as to the source. Many of the earliest printed anatomical figures that were thought to be original are traceable to manuscript sketches that were based upon reading of the anatomical descriptions of the Arabian and of the classical authors. Other printed illustrations based partly on observation show departures from the traditional schemes. There is,

however, even in the improved sketches, a mixture of observation and tradition, with a stronger inclination to preserve the traditional than to let go of it and depend on observation.

The date at which sketches of anatomical subjects were first used is uncertain. There is a tradition that Aristotle employed anatomical plates in his teaching, but no remnants of them are known. There are known, however, manuscript illustrations of anatomy dating back to the twelfth century, and furthermore, some of the manuscript sketches of the early part of the fourteenth century have a recognized genetic connection with the earliest printed illustrations of anatomy. For example, Sudhoff has recently published copies of the diagrams used by De Mondeville, about 1304, to illustrate his lectures at Montpellier, and the connection between these pictures and those published by Peyligk in 1499, and by Hundt in 1501 is undoubted. There are other known correlations between manuscript sketches and early printed figures that will be mentioned later in connection with a consideration of the printed sketches.

The earliest printed illustrations of anatomy occur in the *Fasciculus Medicine* of Ketham of 1491, and, from that time to the publication of the *Fabrica* of Vesalius in 1543, there are about one hundred different anatomical cuts. Some of these pictures are duplicated in different treatises so that the enumeration of figures in the different printed books would exceed this number. This statement does not include the seven hundred to eight hundred anatomical sketches of Leonardo da Vinci, none of which were published until much later. In addition to the printed books of the period there were anatomical plates printed and sold separately. To this latter group belong the figure of the skeleton by Richard Helain, printed in Nürnberg in 1493, and its modification, by Grüninger of 1497, the anatomical plates of John Schott of 1517, the plates of Vesalius of 1538, etc.

The pictures of this period are little known to anatomists, accordingly it is the printed illustrations of anatomy from 1491 to 1543 that are to be brought under consideration in this paper. The writer has had for personal examination the printed books containing the pictures referred to with one or two exceptions

that will be noted below. The subject of manuscript illustrations is not attempted, since very few of these have been available, and the references to manuscript sketches are drawn chiefly from the publications of Sudhoff.

In reviewing the old anatomical treatises, points of bibliographical interest emerge, and comparison of the texts brings out some features of interest to scholars. No attempt has been made however to embrace bibliographical notes and textual comparison. The boundaries of the paper are limited to a consideration of the character and quality of the earliest illustrations of anatomy with the further aim to determine to what extent these are based on observation, and to add some comments on the conditions or the time as they affected the development of observation in science. The pictures are not comprehensive enough in their range to show all the anatomy of the period, but they are significant in showing the spirit of the time, the dependence on descriptions and the lack of a positive anatomy based on observation.

*Sources.* The printed books published before 1543, that contain anatomical figures are medical treatises, anatomical texts and surgeries. I have had for examination, chiefly in the Surgeon General's Library at Washington and the John Crearer Library at Chicago, the primary sources named below. The books are designated by date and abbreviated title only, since the full titles are often long and cumbersome.

I have examined thirteen copies of the anatomical treatise of Mundinus, *Anatome omnium humani corporis interiorum membrorum*. Of these, seven were published separately and six were incorporated with other writings in the *Fasciculus Medicine* of Ketham. The collection embraced: two copies of the Melerstat edition of Mundinus, published in Leipzig about 1495, 39 leaves, 4°, with one illustration; and one copy each of the editions of G. Lincium, Venice, 1494, 22 leaves, no illustrations; F. Picium, Freiburg, 1507, 23 leaves, no illustrations; J. Adelphus, Strassburg, 1513, 40 leaves, figure of the zodiacal signs as related to regions of the body and a rough sketch of the heart; the large annotated edition of Berengarius, Bologna, 1521, 528 leaves (1056 pages), 21 woodcuts and the extensively illustrated edition

of J. Dryander, Marburg, 1541, 70 leaves, 45 woodcuts, one repeated, and two on one plate. These books are all of quarto size. In addition I have had six copies of the *Incipit Anatomia Mundini* in the editions of Ketham mentioned below.

Six editions of Ketham's *Fasciculus Medicinæ* (or *Medicinæ*) came under observation, all of folio size; Venice, 1495, Latin edition; Venice, 1500, Italian; Venice, 1500, Latin; Milan, 1509, Italian; Venice, 1522, Latin; Venice, 1522, Italian. All these contain woodcuts to be mentioned below.

The plate of the skeleton by Richard Helain, Paris and Nürnberg, 1493, 53 cm. high, from the library of Dr. Mortimer Frank of Chicago.

J. Peyligk, *Philosophie Naturalis Compendium*, containing the *Compendiosa capitis physici declaratio*, which is the illustrated part, Leipzig, 1499, folio, frontispiece and thirteen separate anatomical illustrations in the text.

Magnus Hundt, *Antropologium de hominis dignitate natura et proprietatibus*, Leipzig, 1501, 4°, 120 leaves, 19 figures, one being repeated. Two copies of this rare book came under observation, one in the Surgeon General's library and the other in the library of Dr. Mortimer Frank of Chicago.

Phryesen (Fries, Frisen, etc.), *Spiegel der Artzney*, three copies, the Strassburg edition of 1519, folio, Dutch, 4 figures; Strassburg, 1529, German, 141 leaves, one picture in addition to the illuminated title page, and the same, Strassburg, 1532.

Berengarius (Carpus), his *Commentaries on Mundinus* (mentioned above) Bologna, 1521, 4°, 528 leaves and 21 woodcuts. Three editions of his *Isogogæ Breves*: Bologna, 1523, 4°, 80 leaves, 23 figures; a small pocket edition, 1530, 132 leaves, with 24 very crude, small woodcuts copied from the edition of 1523; Venice, 1535, 4°, 63 leaves, 19 cuts.

Petrus d'Abano, *Conciliator differentiarum philosophorum*, 1526, containing the first printed pictures of the abdominal muscles copied from the edition of 1496.

Leonardo da Vinci, *I Manoscritti di Leonardo da Vinci della Reale Biblioteca di Windsor*, etc., Paris, 1898, 1901, etc.; ten of the twenty-four volumes contain anatomical sketches and

notes, 223 plates and upwards of 750 figures, with an introduction by Duval. These anatomical illustrations, executed about 1510, are in all particulars the most notable contribution to anatomy before Vesalius.

J. Dryander, *Anatomia Mundini*, and other old writers, Marburg, 1541, 70 leaves and 44 illustrations.

W. H. Ryff, *Anotomi* (very long title), 1541, woodcuts.

For collateral reading the treatises of Choulant, Chievitz, Hopf, Hyrtl, Roth, Pagel, Sudhoff, Töply, Weindler and Wieger have been of especial service. In Wieger are found photographic reproductions of visceral dissections from Reisch's *Margarita philosophica*, 1503 and 1504, forming a link in the development of anatomical sketches. I am greatly indebted to the contributions of Sudhoff for general enlightenment, for knowledge of the manuscript sources and for an illustrated account of Brunschwig's *Anatomy* in his *Chirurgie*, 1497.

*Mundinus*. (Mondino, etc.; the Romanized form of his name is used here because his book was chiefly printed in Latin.) The anatomy of Mundinus (*Anatome omnium humani corporis interiorum membrorum*, *De omnibus humani corporis interioribus membris anatomia*, *Incipit Anathomia Mundini*, etc.), although not the first treatise on anatomy to be illustrated, is the natural starting point for a consideration of pre-Vesalian anatomy. Appearing, in manuscript, in 1316, it was the first professional treatise on anatomy after more than eleven centuries of Galen. On account of the extensive use in medical schools it forms the genetic link between the ancient anatomy and that of the renaissance period. It was the forerunner of the anatomical treatises that appeared before the epoch-making book of Vesalius.

Mundinus, on account of the influence of his teaching and of his treatise, looms large in the background of historical anatomy. He helped to overcome the opposition to dissection and he is usually credited with having brought the practice into general recognition. Although he was a pioneer in the restoration of anatomy, his way had been prepared by others. De Mondeville as early as 1304 had been illustrating his lectures on anatomy at Montpellier; the Senate of Venice had decreed in 1308 that a

body should be dissected annually; William of Salicet, Richardus and others had dissected before Mundinus.

The purpose of his book was to simplify the teaching of anatomy and it was designed primarily for his students. (As he says: "proposui meis scholaribus in Medicina quoddam opus componere.") It was so highly esteemed that it had a general use for upwards of two centuries and often was used as an introduction to Galen or in connection with his anatomical writings. It came to be prescribed by legislation as the required textbook of anatomy in Italy. Before the invention of printing it was copied and extensively circulated among medical students. Mundinus was a great favorite with the students who came under his instruction. He seems to have been a man of engaging personality gifted with powers of clear exposition. His book is well arranged and terse in description. Although he states that prior to its composition he had dissected three human bodies, it is too much to say that it was an original treatise based on personal observation. He merely brings into systematic form the teachings of Galen with some modifications of his own. Roth and others have pointed out that in his compilation he did not make use of a pure text of Galen in the Greek, but, on the contrary, employed impure Latin and Arabic translations. He does not succeed in overcoming the influence of tradition and of dialectic compilation. With Galen he enumerates five lobes in the liver and perpetuates other errors that observation on the human body should have corrected. His book is also burdened with the terminology of the foreign texts; the stomach, for illustration, is designated the myrach, the peritoneum as the cyphach (siphac), the omentum as zirbus, the mesentery as eurachus, etc., etc. The key to the influence of the book of Mundinus is not its originality but its wide circulation; it is conspicuously lacking in evidences of independent observation.

The book was first printed in small folio form in Padua, in 1478, and, between that date and 1580, when the last edition was published, not less than twenty-five editions are known. These are usually annotated and commonly in quarto form. The thirteen editions of Mundinus examined, excepting those in Ketam,

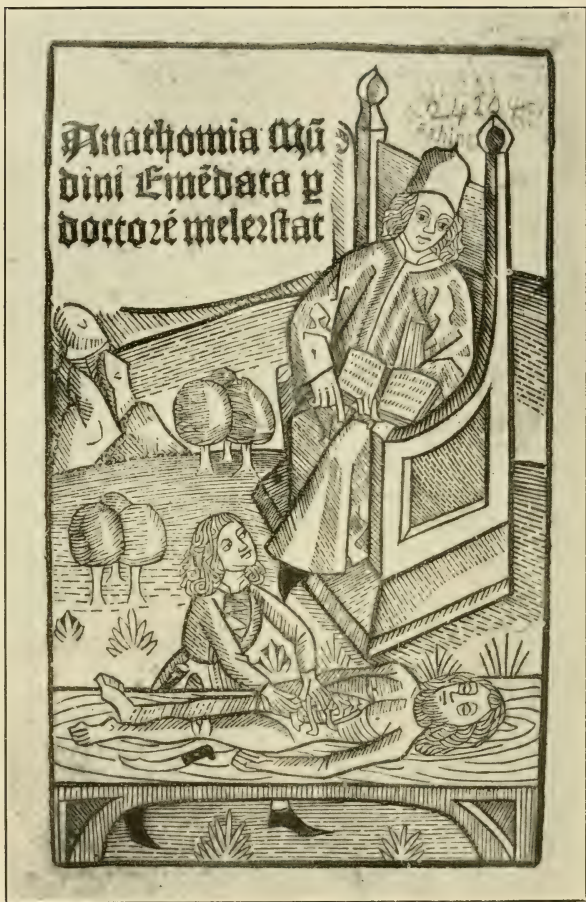


Fig. 1 From the Melerstat edition of Mundinus, Leipzig, 1493

vary from an edition of 22 quarto leaves to the extensive edition of Berengarius, of 1521, containing 40 commentaries and 586 leaves. The 21 illustrations in the last mentioned will be considered under Berengarius.

The only edition to be mentioned at present is that of Dr. Melerstat, printed in Leipzig about 1493-95. The book was published without date or indication of place. The copy in the Surgeon General's library at Washington has a note saying that it was published, probably in Leipzig about 1493. It has 39 leaves, including the title page, with a letterpress of  $3\frac{5}{8} \times 6$  inches. This was the first edition to be printed with a woodcut which is shown reduced in fig. 1. The original is  $3\frac{3}{8} \times 8\frac{5}{8}$  inches. It represents a teacher of anatomy seated and reading from a textbook, while, in front, his demonstrator is engaged with a visceral dissection. The sketch of the viscera is highly diagrammatic. On the table is seen the large curved knife like that exhibited in the pictures of surgical instruments of the period. This picture shows the method of teaching anatomy at that time. Often the reading was done without any subject before the hearers, at other times dogs and other animals were used for demonstration, and, on rare occasions, a human body was dissected in public anatomies. This picture is a type of many others found both in manuscripts and in early printed books. Sometimes in these pictures students are shown grouped around the dissecting table, but the teacher is always seated and reading from a text. The academic dress is a feature of them all and affords an index to the costume worn by teachers and students at different schools and at different periods of time. For several similar pictures see Choulant, Chievitz, etc.

*Kerham.* (Johannis de Ketaz, etc.) The Fasciculus Medicinæ (also Medicinæ) of J. de Ketham is believed to be the first printed medical treatise to be illustrated. The first edition printed in Venice in 1491 contained six woodcuts and the subsequent editions contain usually nine or ten. Prepared under various editors, there are several editions but they are similar as to text and illustrations. The book is of folio size and is a collection of medical writings embracing sections on the means of recognizing

diseases by the various colors of the urine; the practice of venesection and blood letting; comments on surgery; the figure of female anatomy, showing a foetus in the uterus; advice regarding diseases and, in 1493 and thereafter, the anatomy of Mundinus.

Only two of the illustrations can be classed as anatomical, that showing the location of the viscera in the female (fig. 2), and preparation for opening the body cavity, first introduced in 1493, in connection with the *Incipit Anathomia Mundini*. The other illustrations show: the circle of 21 urine glasses, with circles indicating the four temperaments; the signs of the zodiac as related to parts of the body, as in the figures in the old almanacs; the points on the body for blood letting; a sick man on a couch; the wounded man, showing cuts, impact of clubs, etc. The drawings were made by Petrus de Montagnana.

Fig. 2, reduced from the edition of 1491, gives a fair conception of the quality of the pictures. This figure is borrowed from Wiegner, since I have not had the edition of 1491 for examination, but have examined the corresponding figure in various editions beginning with that of 1495. The sketch shows in outline the position of the viscera; the uterus is represented as opened and containing a foetus. In 1493 the drawing of the female figure was modified by observations, and after that date the illustration bears the inscription 'Tratta dal Natura.'

A very interesting connection between the printed copies of this book and its manuscript sources has been brought to light by Sudhoff. He found about 1907, in the Bibliothèque Nationale at Paris, a neatly written Latin manuscript of quarto size, and 54 leaves, which belongs to about the year 1400. In this manuscript is a complete series of the Ketham pictures of 1491, and much of the Ketham text. After folio 45 in this manuscript is an anonymous treatise that agrees substantially with the *Fasciculus Medicine* of 1491. The text and figures of the Paris manuscript are not assembled as in the Ketham of 1491, but the text is in places identical, and the printed figures are evidently copies of the manuscript sketches. The way in which this collection of writings came to bear the name of Ketham is a matter of conjecture. Sudhoff thinks likely that there was a *Johannis*

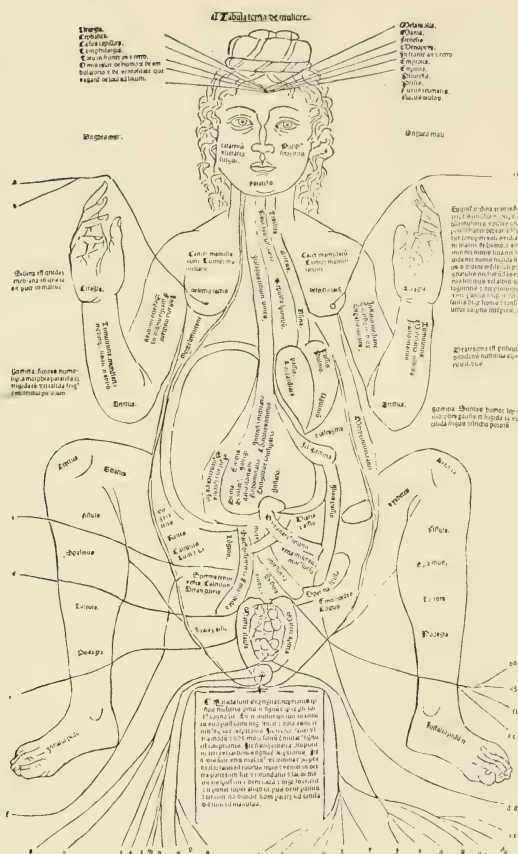


Fig. 2 From Ketam's Fasciculus Medicine, Venice, 1491 (after Wieger)



de Ketham, who, about a century before 1491, assembled the drawings and text, and, that when printed for the first time they bore his name, but of this we have no certain knowledge. This whole collection is probably derived from earlier manuscript sources in French, German and Italian.

Prior to the publication of Ketham, there was printed in 1485, in *De proprietatibus rerum* of Bartholomaeus Anglicus, a woodcut of some anatomical interest. Standing in front of a walled garden is the figure of a man with the abdominal cavity opened and a very diagrammatic representation of the viscera. Within the garden the figure of Eve is appearing before the Lord from the side of the sleeping Adam.

*R. Helain.* Anatomical figures on separate plates were published as early as 1493, the first one to appear being a representation of the skeleton. It is probable that plates of this kind were exposed in barber shops and bath establishments, and that they were also purchased by medical practitioners and by the curious of the general public. A cut of the earliest known picture of this kind is shown in fig. 3, which is copied from Wieger, although I have since seen a copy of the original in the library of Dr. Mortimer Frank of Chicago. It is attributed to a Paris physician, Richard Helain, and was printed in Nürnberg in 1493. Whether or not it was also printed in Paris is not known. The original plate was 53 cm. high. It seems to have been drawn from a partly dried specimen and the drawing is in many particulars fantastic. Among the curious features are, the dark abdominal portion, the expanded pelvis, the divided lower jaw and numerous teeth (17 on the lower jaw), the bones of the feet and the 'os laude' of the skull. This 'os laude' or 'os capitale relaude' is an apochryphal bone, and its designation will puzzle those acquainted with classical Latin not a little. We might expect it to be *os laudis* but in the corrupted Latin of the period the termination *e* is commonly used for *ae* and we conclude that it is 'os laudae.'

This anatomical plate is referred to by Hyrtl, Wieger, and others as the work of Ricardus Hela. There is probably a mistake in the name, since Sudhoff, by a careful search of the records of the Paris physicians of this time, was not able to find the name of

Hela, but instead found that of Richard Helain. The plate should probably be attributed to him. This picture formed the basis for a modification by the publisher Grüninger in 1496-97 (fig. 4) which was printed in Brunschwig's *Chirurgie*, in 1497, and in various other texts. The picture (fig. 4) is however taken from Phryesen's *Spiegel der Artzney*, 1519.

The earlier manuscripts show a considerable number of sketches of skeletons, some of which resemble the drawing of Helain. (See Sudhoff, *Studien zur Geschichte der Medizin*, Hft. 4.)

*Petrus d'Abano*. In the *Conciliator differentiarum philosophorum* of Petrus d'Abano there appeared in 1496 the first printed illustration of the abdominal muscles. This is shown, considerably reduced, in fig. 5, which is taken from a reproduction of the woodcut by Sudhoff in its original dimensions ( $5\frac{5}{8} \times 6\frac{7}{8}$  inches). I have had for examination the 1526 edition of the *Conciliator* in which the same figure occurs, slightly modified and reduced in size. In that edition, in the 199th differentia, on page 231, is a description of the eight abdominal muscles. The picture is evidently made with the help of a dissection. There were earlier editions of the *Conciliator* in 1472, 1476, 1483 and 1491, but there is no picture in any of these; the first illustrations occur in the third Venetian edition of 1496. There is also a manuscript edition of the *Conciliator* near the beginning of the 14th century that speaks of eight abdominal muscles from Greek and Arabian sources.

Sudhoff, in pointing out that the figure of 1496 was based on a dissection, locates that dissection in Bologna. He found a copy of Mundinus with a marginal pen drawing of these muscles by a student, dated Bologna, 1494.

*Brunschwig*. In the interval between 1496 and the appearance of Peyligk's illustrated treatise came the publication of Brunschwig's *Chirurgie* in 1497. A few pages of this is devoted to anatomy, and in it we find a picture of the Grüninger skeleton (fig. 4), which was a modification of the Helain skeleton of 1493, and also a picture of the wounded man showing visceral anatomy. This picture is one of the series showing the development of illus-

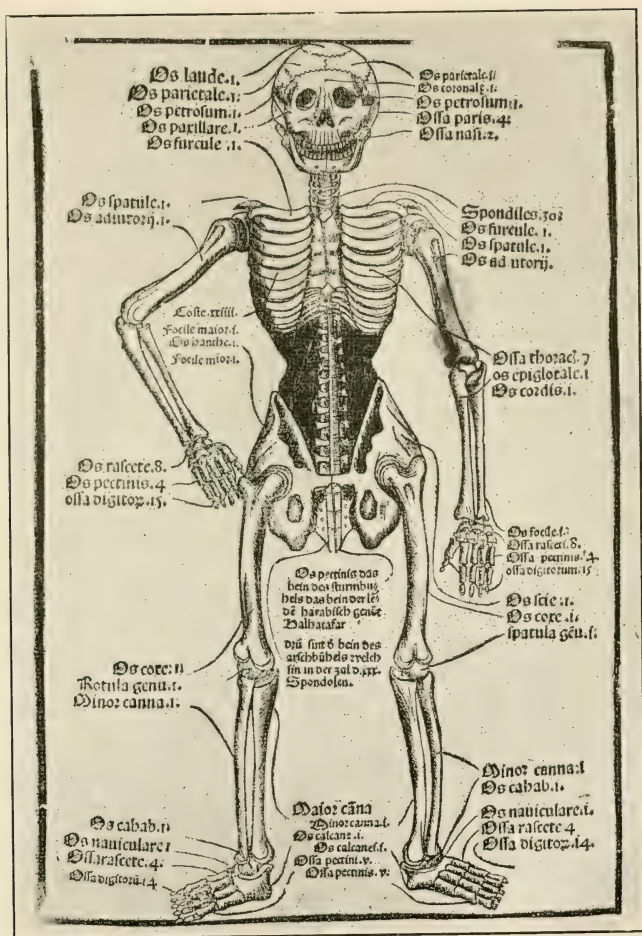


Fig. 4 The Grüninger modification (1497) of the skeleton of Helain. Printed in Brunschwig's *Chirurgie*, 1497, and in other later texts. This cut from Phryesen's *Spiegel der Artzney*, 1519

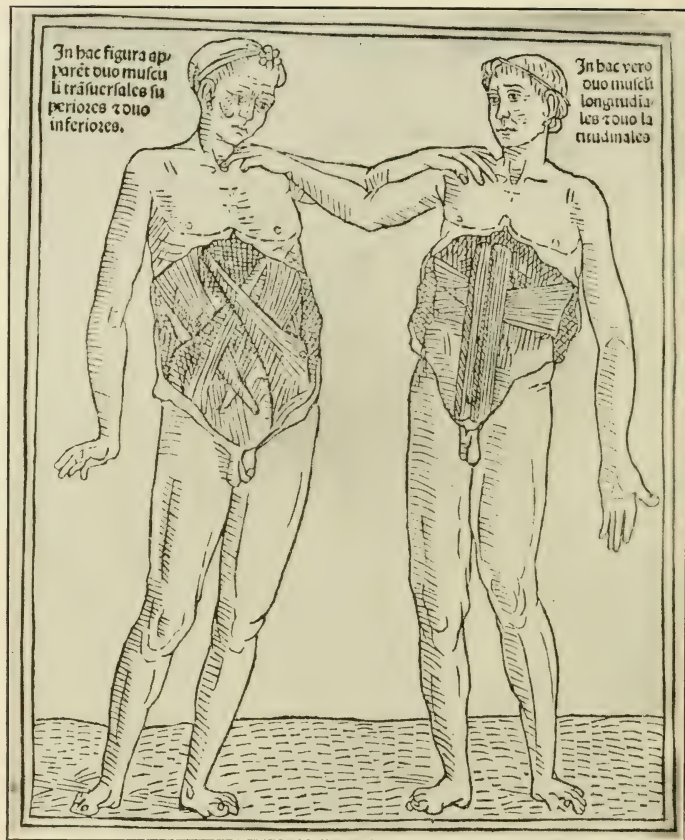


Fig. 5 First printed sketch of the abdominal muscles, from the *Conciliator Differentiarum* of 1496 (after Sudhoff)

trations of internal anatomy. It is reproduced by Sudhoff in the *Archiv für Geschichte der Medizin*, Bd. 1.

*Peyligk.* In 1499 was published the *Philosophie Naturalis* of Johannes Peyligk which contains ten figures of separate organs of the body besides one large figure showing internal anatomy of head, thorax and abdomen. Peyligk's book is the compilation of a jurist of Leipzig. It is a fine folio of 96 leaves,  $8\frac{1}{2} \times 11\frac{3}{4}$  inches, with the letterpress  $4\frac{3}{4} \times 8$  inches. The last twelve pages are embraced under the title *Compendiosa capitis physici declaratio principalium humani corporis*, etc., and it is this part alone that contains the anatomical illustrations. The frontispiece, which is printed on the reverse of the last page of the *Philosophie Naturalis*, is shown reduced in fig. 6, the original being  $3\frac{1}{2} \times 7\frac{5}{8}$  inches. In this figure we see the three cavities (venters) of the body indicated; the upper (supremis), containing the animal members; the middle (medius), containing spiritual members and the lower (inferioris), containing the natural members. The head shows only the ventricles of the brain as conceived of at that time. The thoracic cavity has a diagram of the lungs, the heart, the trachea and the œsophagus. Below the diaphragm, which is indicated as an oblique line passing across the trunk, there is represented the stomach, the spleen, the intestines and the liver with two blood-vessels. The liver is represented with five lobes according to Galenic tradition, and the gall-bladder is shown as a pear-shaped vesicle on the liver. In addition to this large diagram of the organs *in situ*, the text is embellished with sketches of the separate organs. Fig. 7 shows a picture of the page containing the figure of the stomach, œsophagus and intestines. Fig. 8 shows the separate illustration of the heart; the manuscript notes in this copy are also to be seen. All these figures, manifestly, are diagrams and not sketches from nature. Since they are the earliest printed illustrations of separate organs, it is an interesting matter to locate their source. Are they purely fanciful sketches based on descriptions of earlier writers?

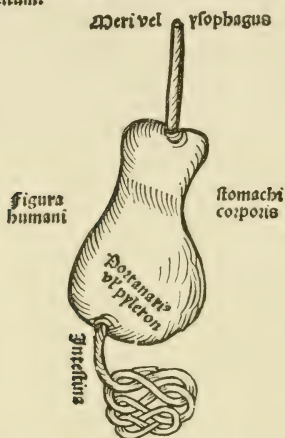
The source of Peyligk's figures remained for a long time undetermined, and the assumption was generally made that they were schematic mental pictures, derived from reading the anatomical

descriptions of Arabian and classical writers, and transferred to paper. The sketches are certainly schematic and show the influence of tradition but they were not produced by Peyligk. The speculation of Stockton-Hough that they came from an illustrated Mundinus of 1498 is unfounded. There is no known illustrated Mundinus of 1498 and the suggestion is probably due to a confusion of the Mundinus text in Ketham's Fasciculus of 1495. Several of the sketches are now traceable to the diagrams of Henri de Mondeville, and used by him about 1304 in illustrating his anatomical lectures at Montpellier. Pagel made known in 1889 that de Mondeville had employed sketches and, in 1890, Nicaise reproduced the miniature sketches of entire figures showing internal anatomy. He says that de Mondeville also made use of sketches of separate organs of which all trace had been lost. These separate sketches have now been unearthed and were published in 1908 by Weindler, and, in a separate article, by Sudhoff. Those reproduced by Sudhoff embrace eighteen manuscript figures, nos. 1 to 7 found in the Royal Library at Berlin and nos. 8 to 18 in the Royal Library at Erfurt. The resemblance of some of the figures of Peyligk to these manuscript sketches of de Mondeville, leaves room for no reasonable doubt that the latter were the sources from which the Peyligk figures were drawn. It is uncertain how the pictures of de Mondeville originated. Sudhoff suggests that possibly de Mondeville began illustrating his earliest lectures at Montpellier by making diagrams of traditional anatomical sketches. Of this we have no certain knowledge, but we have, at any rate, the sketches of separate organs of de Mondeville to add to his miniature pictures of entire anatomical figures that were previously known.

*Hundt.* The next printed anatomical illustrations to come under notice are those of Magnus Hundt, a Leipzig anatomist. His *Antropologium de hominis dignitate*, etc., published in 1501, is a rare quarto of 120 leaves, with a letter-press  $3\frac{1}{4} \times 5\frac{3}{4}$  inches. It contains nineteen illustrations, one of which is printed twice. The sketch of the viscera *in situ* is shown in fig. 9. There is another large figure in the book (the one that is repeated) showing the ventricles and the general location of physiological function



do. vt digestio sua confortaret. Caro enī est calida z humida. Ex quibus du-  
pendet v̄tus digestiue. in aīali Rotundus vt sua rotūditate cibū reciperet  
plioiē. z vt mali humores in eo generati facilius possint desiccari. Oblon-  
gus vt facilius cū supioribz z inferioribz iungere. Latius vero inferius. quia cū  
mo sit erecte figure. cibus ex sua grauedine semp descendit. Nervosus sup  
ad vigoandū appetitum.



¶ Stomach⁹ vt dī Constantin⁹ epate circūdat. vt ei calor maior ad ciboz ꝛ  
ctionē ab epate administrat. Epas enī suis qñqz pēnulis stomachū circūcti  
et ei calorem tribuens succositatē z humore vnde sanguis gīatur per quas  
venas mesaraicas recipit z fortiori caloris actione in sanguinē alterando  
uertit. Dixi ad ciboz decoctionez expumendo ppriā operationē Et officiū  
machi. q: stomachus est tot⁹ corpis passifamilias. oīni mēbroz nutrimentū  
piēs. Et singuloz mēbroz put expedit administrat necessitati. Cōponit aut  
machus ex duabz tunicz sine pelliculis. Vna est interior. q̄ est subtilior z q̄

Fig. 7 Part of page from Peyligk, showing sketch of stomach and intestines, 1499

of the brain. The original of fig. 9 is  $3\frac{1}{8}$  x  $5\frac{1}{4}$  inches. It is in some particulars more crude than the corresponding figure of Peyligk. In the thoracic cavity one sees the undivided lung, the heart, the trachea and the oesophagus. In the abdominal cavity is the large many-lobed liver with the gall bladder on its surface, the pouch-like stomach, the spleen, the loops of the intestine, and, pushed to one side, the kidneys, the bladder and the testes. The blood vessel connected with the liver is the 'vena chilis' and the blood vessels to the kidneys are the 'venæ emul-

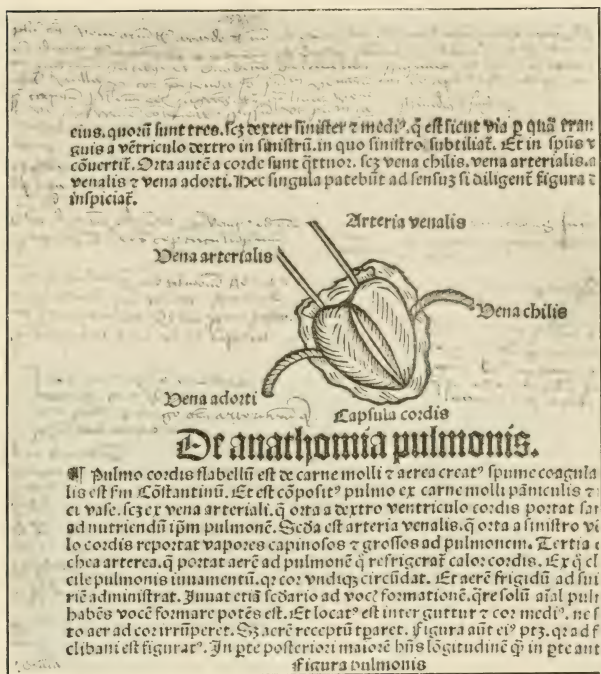


Fig. 8 Sketch of the heart from Peyligk, 1499

gentes.' In the figure that I have photographed the iris of the eye is black, while in that reproduced by Sudhoff from the copy in the Leipzig library the iris of the right eye is indicated by a white circle.

The book is provided with text-figures of separate organs copied from Peyligk's treatise. The figures, however, are not printed from the same blocks; they are nearly identical but careful inspection will show slight differences in the lines. Fig. 10 shows the sketch of the liver with a part of the text. It is the same as the corresponding figure in Peyligk but is not quite so carefully engraved.

*Gregor Reisch.* In the 1504 edition of Reisch's *Margarita Philosophica* there is an illustration (see fig. 11) showing new details in internal anatomy of the thorax and abdomen. Although the anatomy is very crude it is an improvement over the corresponding figures of Peyligk and Hundt. The kidneys and bladder are represented in a more nearly normal position. The lungs are

**Figura de situ viscerum.**

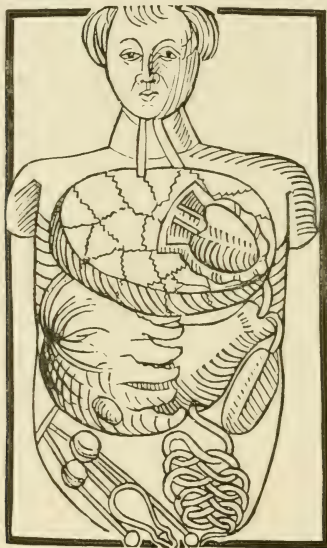


Fig. 9 Visceral anatomy from Hundt's *Antropologium*, 1501

divided into lobes; the liver, stomach, spleen and intestines are still very untrue to nature. The nomenclature of the period is shown in the names attached to the organs, the lung, 'pulmo,' the heart, 'cor,' the liver, 'epax,' the kidney, 'ren,' the bladder, 'vesica,' etc. In 1503 a similar picture had appeared in the edition of the *Margarita Philosophica* from the printing house of

**Pli. xliij. natura**  
**lis his. c. j. in fi.**  
**Auricula. ij. can. c.**  
**xliij.**  
**vi. apbor. xlvij.**  
**ij. apbor. c. xliij.**

**Alber. xliij. de aia**  
**lib. c. vij. in fi.**

**Constantinus**

**Auric. xliij. tercij**  
**c. j.**  
**gal. v. d. iura. c. ij.**  
**ij.**


**Nicolas. fmo. v.**  
**trac. v. c. j.**

cit. aq. lactis. aristo ogia rotunda cu aq. bibita. Lactis crudus cum  
 aceto sumptus. corallus cu melle sumptus. orimel. rē. Duris apantibus  
 et duritiei splenis. acetosa cu aceto emplastrata. agn. castus i vino  
 forti coctus. et cu cera et oleo vnguentus factus valde pferit. aristologia  
 cu aqua rē. Qui frequenter incidit catharrum non sunt spleneticis. Sple  
 ticus si supuenerit dissenteria boni. Autum. inimic. est spleneti  
 cis. Dine aial. hns pui splenē etia habet pui pulmonē. q. par. pos  
 tat de aqua. hns aut magnū pue est crassitiei q. sit ab epate qd cum  
 magnū est et magne hybris impedit attracco splenis. E par. et splen  
 sunt duo postea quibz vene sunt infixe ex vtraq. pte. ij. de ptribz. vij.  
 Repleti cibo maiore sentiunt dolorem propter compressiōem splenis  
 Splenetici est color. sedus declinās ad liuiditatem rē.

**De epate et ptribz eius caput. liij.**

**Par est membrū organū**  
**unicū primū sanguis in**  
**strumetaliter gnatū.**  
**carne sanguine a villis**  
**neruoz vacua et venulis ptextus**  
**magnū. p. cauū. rubicundū. et lu**  
**bicosum. in dextro latere aialis**  
**collocatū stōacho suprepositū. ut**  
**suo calore primā iunet digestiōe**  
**Epar esse membrū organū do**  
**cet diuersitas formaz in pibz a**  
**toto. Non em qlibet ps ei? inte**  
**gralis toti? capit denoiationem**  
**Tameti pēnule ipsi? epar intri**  
**sice pstitueres. p. cauitatē et gibbo**  
**sitatē habet eandē cū toto. nō tū**  
**isti? q. a nomē totus merentur.**  
**Epar aut latine dicitur secur quasi ia**

**Uena porte** **Epar figura**



**Uena bilis**

Fig. 10 Part of page from Hundt's Antropologium, 1501

John Schott. In this earlier picture the ureters are not shown, and the intestine is represented as connected with the bladder. I am indebted to Wiegner's treatise for the sketch of 1504, that appeared in the *Margarita Philosophica* published by Grieninger. This figure was reproduced in other texts and the original of this cut (fig. 11) is in Brunswick's *Destillirbuch*.

Leonardo da Vinci. With Da Vinci we come to the one man who, before Vesalius, showed independence in observation and

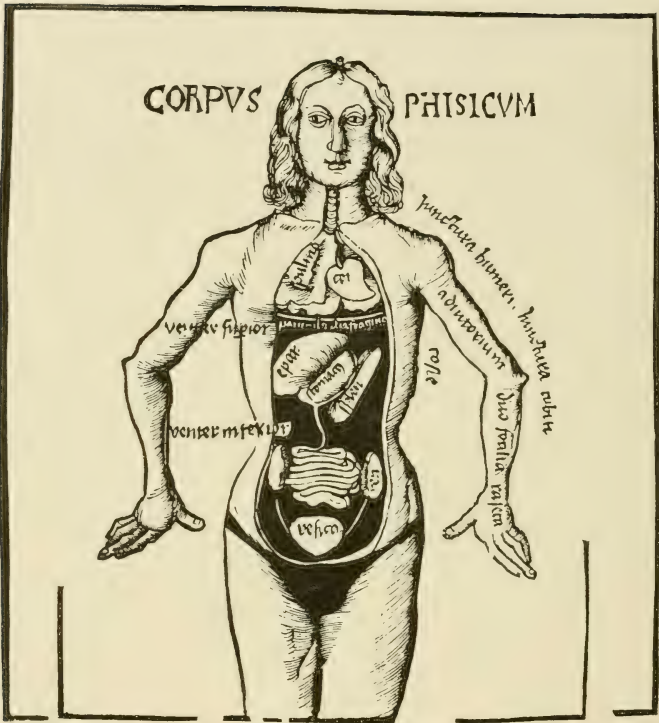


Fig. 11 Visceral anatomy from Reisch's *Margarita Philosophica*, 1504. This cut from Brunschwig's *Destillirbuch*, 1512 (after Wieger)

notable fidelity to nature in his sketches. Although the larger number of his anatomical drawings were made about 1510 they were not fully published until 1898 and 1901. They bear internal evidence of having been made from actual dissections. It is well known that he became associated with Della Torre who projected a treatise on anatomy for which Leonardo was to supply the drawings. Nevertheless, Da Vinci had studied anatomy independently before his association with Della Torre, and he contin-

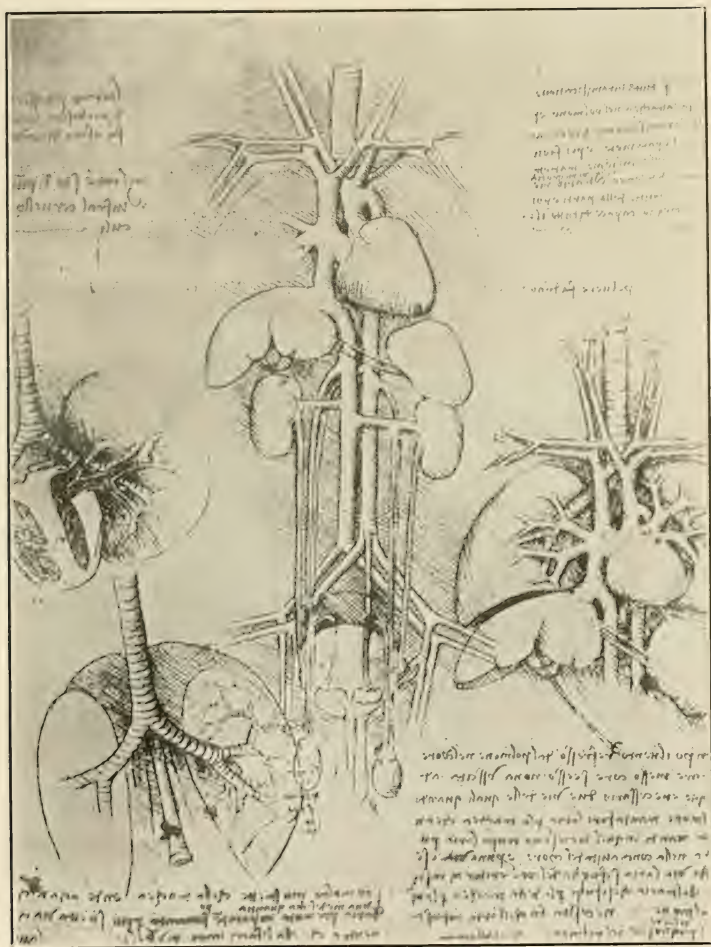


Fig. 12 Anatomical sketches from I Manoscritti di Leonardo da Vinci, 1510 (after the Paris facsimile edition)

ued to make dissections and anatomical sketches after the death of the latter in 1506. We may assume that his method was improved by intimate collaboration with a professional anatomist, but we must recognize that this extraordinary man was a master unto himself. The association with Della Torre was not merely that of an artist working under an anatomist who exposed the parts and required sketches made under his direction. It was

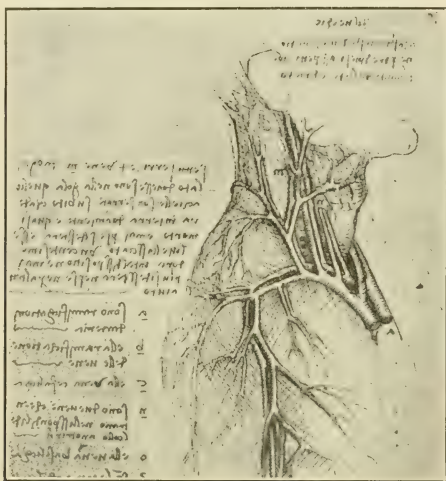


Fig. 13 Anatomical sketch by Leonardo da Vinci, 1510

rather the coöperation of two anatomists, one of whom was gifted with great powers of artistic delineation. Antonio de Beatis had it from Leonardo's own lips, about 1510, that he had dissected not less than thirty human bodies, both male and female.

Leonardo projected a comprehensive work on anatomy of which he speaks in his History of Painting, and also in his manuscript notes. The notes and drawings bear testimony that this treatise was not designed merely for artists but was to be, as well, a work for medical students and for the professional anatomist.

The working drawings and notes for this projected work are preserved as a part of the manuscript collection in the Royal Library at Windsor Castle. They were published in Paris in 1898 as Foglio A of Leonardo's *Manoscritti*. This sumptuous volume



Fig. 14 Anatomical sketch by Leonardo da Vinci, 1510

contains 245 anatomical sketches, reproduced as fac-similes both as regards the sketches and the paper upon which they are drawn. The notes are translated into French. His other anatomical sketches, also in the library at Windsor Castle, were published

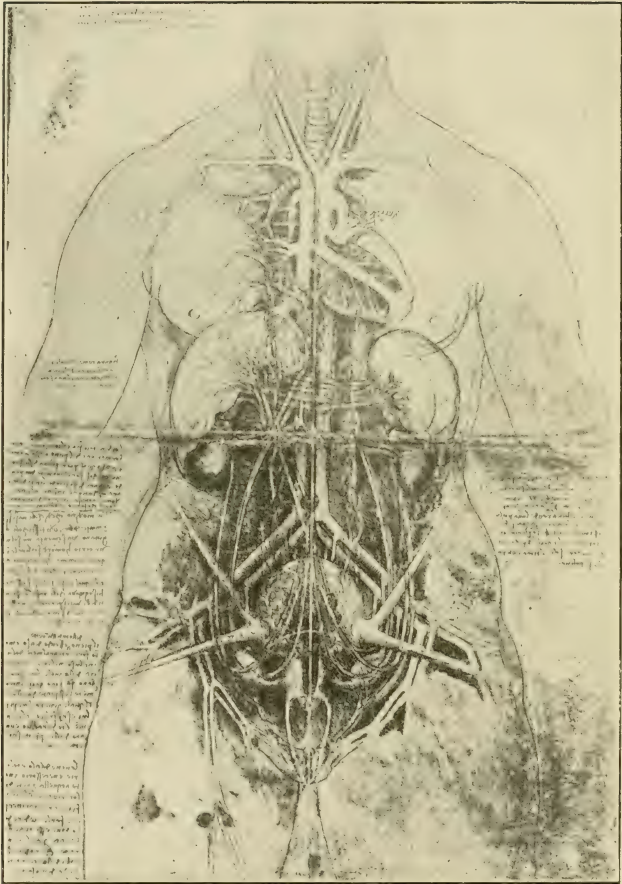


Fig. 15 Anatomical sketch by Leonardo da Vinci, 1510

in eight additional volumes in 1901. This does not include the volume on the anatomy of the horse. The range of the drawings is astonishing; the entire collection embraces more than 750 separate sketches, some of them being several times repeated. The notes accompanying the sketches, always written from right to left, are, usually, descriptions of the figures, but, sometimes, are general reflections regarding the plan of his projected book. That he read anatomy is evident since he specifically corrects some misstatements of Mundinus. Leonardo placed great reli-

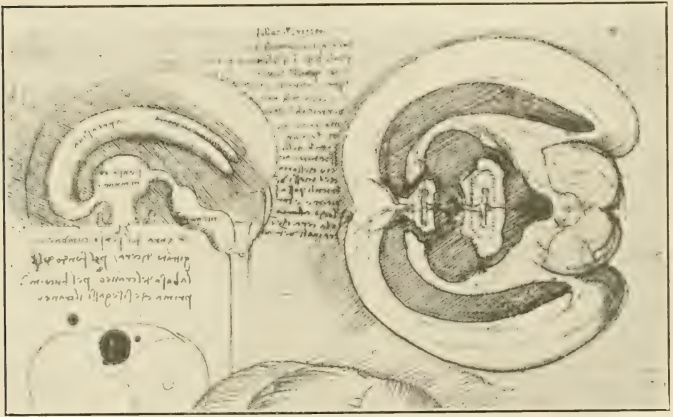


Fig. 16 Sketches of sections of the brain by Da Vinci, 1510.

ance on good figures, declaring them to be essential to the understanding of anatomy. Some of his delineations of muscles have been so frequently reproduced that they are well known, but it is not so generally known that he made deep dissections of all kinds including the viscera and the brain.

The reproduction of a few of Leonardo's sketches will serve to show their quality, and will at once reveal the fact that they are totally different from any other sketches of the period. These drawings are not made from anatomical descriptions, but from



Ein cōtrafact Anatomy d'innerē gliedern des  
mēschē durchs H. hochgelehrte phisici vñ medicine Doctor Wēdelinū Baer vñ Dr.  
Johann zu Straß. declariert in beuēse vñer scherer wūdarzge grūlich durchsuche

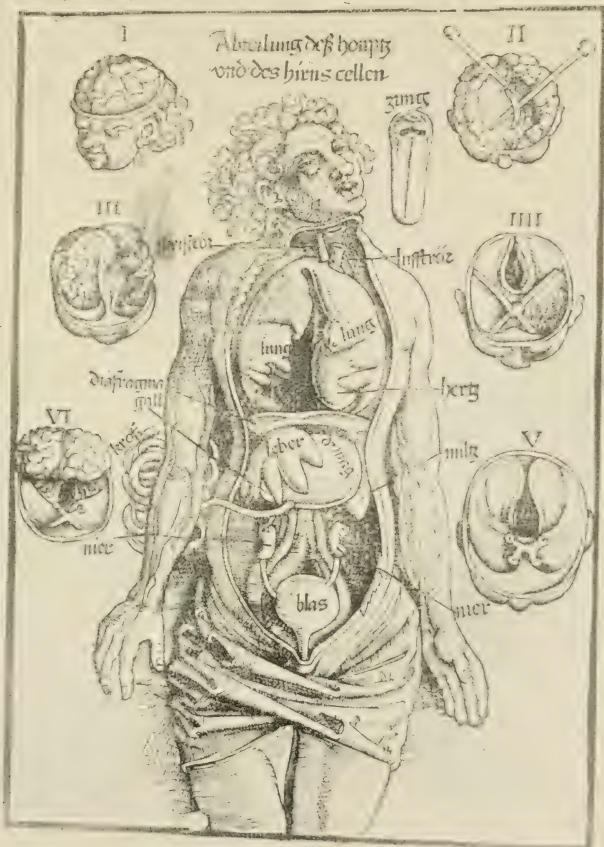


Fig. 18 Anatomical sketches from Phryesen's Spiegel der Artzney, 1517. This cut from a Dutch edition of 1519



Fig. 19 Title page of the Commentaries on Mundinus by Berengarius, 1521



Fig. 20 Figure of the skeleton from the *Isagogæ Breves* of Berengarius, 1523

**I**n ista fi-  
gura vident  
ossa ptis po-  
sterioris hois  
videt et due  
calue: in qru  
dextra ut co-  
ronalis qmis-  
sura q e i pte  
superiori: ut &  
sagittalis: q e  
i medio: ut et  
laude comis-  
sura: q e i pte  
inferiori: a late-  
ribus et videt  
due commissu-  
re a me supra-  
noiate in ana-  
tomia cranei  
q sunt supra  
comissuras sq-  
mosas exites  
ppter aures: s-  
iste sunt fere  
insensibiles: a  
sinistris e alia  
calua: in q ui-  
det mandibu-  
le: & pars co-  
missure coro-  
nalis: & due  
comissure in  
fra sagittale  
ad unum latus  
existentes: &  
ut unum os de  
duobus ossibus  
paris: qd est a regione oculi seu ab osse dictu pomu faciei tendens p latu ca-  
pitis uersus aurem.



Fig. 21 Posterior view of the skeleton from the Commentaries on Mundinus of Berengarius, 1521

Fig 13 shows the chief blood vessels of the neck and the adjacent region.

Fig. 14 shows a deep dissection of the blood vessels of the thigh.

In fig. 15 we have a rather comprehensive dissection of the thorax and abdomen with the alimentary canal removed. The original of this figure is  $13\frac{1}{2} \times 22$  inches.

A limited number of drawings can give no adequate conception of Leonardo's work in anatomy. His sketch of the stomach and intestine is a good drawing of the relative size and the normal arrangement of these viscera. In the delineation of muscles it is not merely the superficial layers that engage his attention, he shows details of the arrangement of the tendons on the toes and fingers, a number of cross-sections of the leg at different levels, the muscular architecture of the heart, etc. Among his many pictures of the bones, he correctly draws vertebræ from various aspects, and the bones of the fore-arm in pronation as well as in other positions. He made sketches of the dissection of nerves. His figures on generation show uteri opened, with contained fetuses, and the placental connection.

Before leaving his work, however, we should have one of his sketches of the brain as shown in fig. 16. Here one sees, on the left, a median sagittal section, and, on the right, a horizontal section. These sketches show fairly the extent to which the brain had been dissected up to the year 1510.

Other contemporary or nearly contemporary artists, as Michael Angelo, Raphael, and Albrecht Dürer, made anatomical sketches, but not so comprehensive as those of Da Vinci, and the details regarding which it is not necessary to consider.

*Johannes Schott.* In 1517 there appeared from the publishing house of John Schott at least two anatomical plates, one representing a skeleton and the other a sketch of the internal anatomy of the body. The picture of the skeleton is shown in fig. 17. It is still very crude in its execution, but in some particulars is an improvement on the earlier printed figures. The skull is better drawn than in the plates of Helain and Grüninger (figs. 3 and 4), but it still shows the spurious 'os laude, sive capitale.'

There are other marked deficiencies as in the arm and wrist, where the carpal bones are enumerated as eight, but are not drawn, etc., etc. The sketch of the internal dissection was published in several texts as in the Phryesen of 1518 (see Chievitz, p. 90), 1529, etc. Choulant reproduces a similar but not identical figure, also from Phryesen, the plate of which bears the date 1517.

*Phryesen.* (Fries, Friesen, etc.) In 1518 there appeared in Strassburg the *Spiegel der Artzney* of Laurentius Phryesen, containing two plates. The one is a copy of the Grüninger skeleton (see fig. 4), and the other a visceral anatomy, surrounded by six figures of the anatomy of the brain and one of the tongue. This cut appears in the different editions of Phryesen with some modifications. Fig. 18 shows a copy of this plate from a Dutch edition of Phryesen dated Strassburg, 1519. The original woodcut is  $5\frac{1}{2} \times 7\frac{5}{8}$  inches. The edition of 1529 contains another picture of the visceral dissection,—the same as shown in Chievitz, fig. 31,—that lacks the marginal sketches of the brain, and is also somewhat different in other details. The figures of the brain in the *Spiegel der Artzney*, except for those of Leonardo, are a new departure in anatomical illustrations.

*Jacobus Berengarius Carpensius* (Carpus). Berengarius has often been heralded as the greatest anatomist between Mundinus and Vesalius, and, if we except Da Vinci, the assignment of this rank to him is perhaps justified. Whatever may be said of his alleged dissection of more than one hundred bodies, the illustrations of Berengarius are not original, nor are they based on good observation. They bear resemblance to sketches in the manuscripts of the fourteenth century and to printed pictures in earlier publications, as the *Conciliator differentiarum* (1496), *Margarita philosophica* (1504), etc. As has already been said, we find that all sketches of the period, with the sole exception of those of Da Vinci, show interrelationships with manuscript illustrations as well as with earlier printed figures. As Roth has pointed out, the anatomical writings of Berengarius are compilations without credit being given to the original sources, and there is inharmony between his text and the illustrations,—a circumstance that is, at times, adverted to by himself. It is altogether likely that the

cuts were inserted by his publisher from such pictures as were available.

The first anatomical publication of Berengarius was an extensive series of commentaries on Mundinus. In this the text of Mundinus is printed in larger type, and the forty commentaries in smaller, but so extensive are the annotations that the book is brought up to a thick quarto volume of 1056 pages. This book, published at Bologna in 1521, is rare and a cut of the title page is shown in fig. 19. The size of the original is  $4\frac{3}{4} \times 7$  inches; the border is red and the enclosed printing black. His commentaries contain at times corrections to Mundinus, and show the results of some observations mixed with dialectic compilations from the earlier writers. In the 21 illustrations the dependence on tradition is very marked.

He soon branched out for himself and wrote an introduction to anatomy, designated *Isogogæ breves*, etc., which was first published in 1522 and followed by a modified edition in 1523 which is the only one well known. In addition to a copy of his commentaries of 1521, I have had for examination three editions of the *Isogogæ breves*; that of 1523, Bologna, 4°, 80 leaves with 23 woodcuts; an edition of 1535, Venice, 4°, 63 leaves, 19 plates, and a small pocket edition ( $2\frac{3}{8} \times 4\frac{1}{4}$  inches letter-press), dated 1530, and containing 24 figures. The illustrations are wretched copies of those of the edition of 1523, the increase in their number, by one, is owing to the separation of two figures that appear on one plate in the larger edition. This appears to have been a relatively cheap edition for students.

More than one-half the illustrations of the commentaries are reproduced in the *Isogogæ* of 1523 and new ones are added. Most of the plates in the edition of 1523 are provided with an ornamental border, added to a double line boundary, while the plates of the commentaries of 1521, are limited by a single line border. Roth reproduces a full-size figure of the skeleton from the *Isogogæ* of 1523, but his plate lacks the ornamental border.

Fig. 20 is a representation of the skeleton from the *Isogogæ* of 1523 in which the ornamental border has been retained, but the marginal description, present in the original, has been cropped

off. This curious figure has 13 ribs, widely expanded pelvis and a spurious fissure in the frontal bone.

The posterior view of the skeleton, shown in fig. 21, is taken from the commentaries of 1521. It has a single-line border and the marginal note has been retained. The basin-like pelvis appears more fantastic than in the preceding figure. The skull shows two spurious furrows on the parietal bones, the presence of which seem to have confused Berengarius. The two best illustrations in Berengarius are those of the bones of the hand and of the foot. The close resemblance of these pictures to drawings of Leonardo da Vinci gives ground for the suspicion that they were in some way based upon his sketches. Although this is a mere conjecture, these two figures are on a different plane of accuracy from any other illustrations in the *Isogogæ breves*.

*Dryander* (also known as Johann Eichmann). This professor of anatomy at Marburg published, in 1537, an *Anatomiae h. e. corporis humani dissectionis pars prior*, etc., illustrated by 20 plates that were based on dissections. I have not seen a copy of this work, but have examined his edition of Mundinus and other earlier writers, published in 1541, which contains most of these earlier figures, some new ones, and 18 figures copied from Berengarius. The copy at my disposal contains 45 figures, one plate of which is repeated. Some of Dryander's illustrations are a considerable improvement on those of Berengarius. Fig. 22 shows his sketch of the alimentary tube, the original woodcut being  $4\frac{1}{4}$  x 6 inches. The drawing of the caecum and the vermiform appendix shows that it is based on observation, but the figure is not so good as that of Da Vinci of the corresponding parts.

*Walther Hermann Ryff*. In 1541 appeared Ryff's *Anatomi* with a very long and cumbersome title. This book, of which I have examined a copy in Dutch and one in German, was published after the first plates of Vesalius (1538) and before the appearance of the famous *Fabrica* (1543). It, with the Dryander mentioned above, lies on the border line of pre-Vesalian illustrations of anatomy. One of Ryff's illustrations of the arterial circulation, reproduced in fig. 23, gives a fair idea of the appearance of his sketches.

Other anatomical illustrations of the pre-Vesalian period embrace the very rare copper plates of Canano, showing bones and muscles of the arm. Choulant says that, prior to 1543, one book

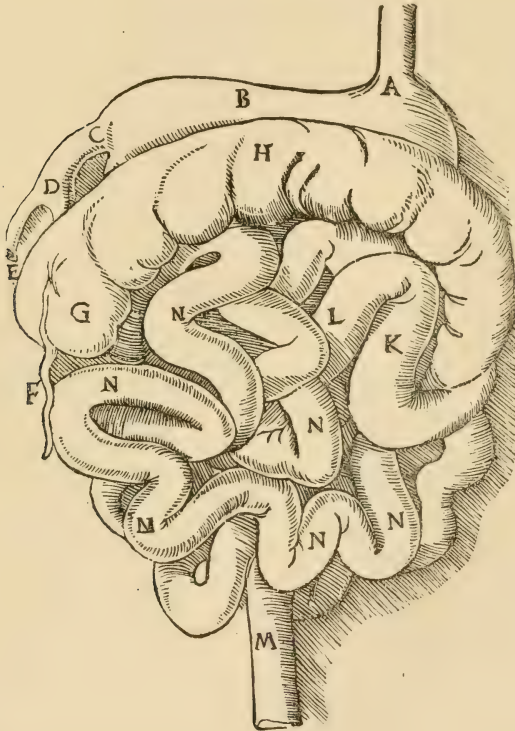


Fig. 22 Anatomical sketch from Dryander's edition of Mundinus, 1541

of the work was published, containing 27 illustrations, but the work was never completed. Between 1536 and 1543 there were several plates of anatomical figures placed on the market. These so-called 'Fliegende Blätter' include the six *Tabulæ anatomicæ*

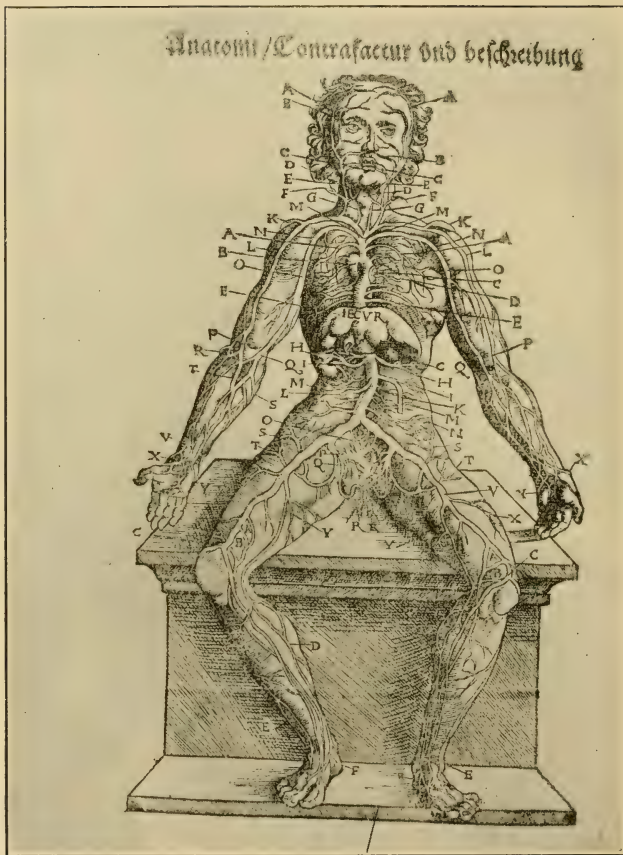


Fig. 23 Anatomical sketch from Ryff's *Anatomi*, 1541

of Vesalius that appeared in 1538 and were a forerunner of his great work of 1543.

There were also during the period other anatomists of more than usual insight, as Achillini, whose anatomical treatises were

not illustrated, and, therefore, do not properly come under consideration here.

*Summary.* The chief printed illustrations of anatomy before Vesalius may now be chronologically arranged, omitting different editions of the same work with slight modifications of the figures:

1491. The arrangement of the viscera in the human female in Ketham's *Fasciculus Medicinæ*.
1493. The skeleton of Richard Helain.
- 1493(?). A demonstration of visceral anatomy in the Melerstat edition of Mundinus.
1496. The abdominal muscles, in the *Conciliator differentiarum*.
1497. Plate of the Grüninger skeleton.
1497. The wounded man with internal anatomy in Brunschwig's *Chirurgie*.
1499. Anatomy of the three body cavities, together with figures of the separate organs, in Peyligk's *Compendicæ*.
1501. Similar illustrations in Hundt's *Antropologium*.
- 1503-'04. Organs of the thorax and abdomen in Reisch's *Margarita Philosophica*.
- 1510 (?). Leonardo da Vinci, more than 750 sketches of human anatomy; not, however, published.
1513. Mundinus, zodiacal signs and rough sketch of the heart.
1517. Plates of visceral anatomy and of the skeleton published by Johann Schott.
1518. Skeleton and visceral anatomy in Phryesen's *Spiegel der Artzney*.
- 1521, '22-'23. Berengarius, commentaries on Mundinus and *Isogogæ breves*.
1536. *Fliegende Blätter*.
1538. Six *Tabulæ Anatomicæ* of Vesalius.
1537. Dryander, *Anatomia, corporis humani*, etc.
1541. Dryander, edition of Mundinus, with 45 illustrations.
1541. Ryff, *Anatomi*.

The survey of these printed sketches of anatomy, covering a century-and-a-half before Vesalius, brings into notice the relatively slow progress. While we remember that this is the period of the awakening of the scientific spirit, still, the drama of intellectual progress does not unfold as rapidly as we might expect. Why, after the revival of dissection under Mundinus, and why, especially, after the introduction of printing, was there not more rapid progress? Some seek to find an answer in the difficulty of getting material for dissection and others in the opposition of the church, but the thing that held anatomical science in check, was not so much the lack of opportunity to dissect as the mental habit of the time. The disposition to dissect was not especially strong. That internal hunger for the analysis of nature at first-hand was not of dominating insistence. The effects of tradition and of education had to be overcome, and the gradual assimilation of new methods and new ideas was necessarily slow. Those who would have done better under gifted and inspired leaders were perplexed and too closely bound by the mental habit of the time to map out and follow an independent course. Thus, the retarding influence was generic rather than specific. Independent spirits of great originality were rare then, as now, and it seems natural that the habit of imitation should have so long perpetuated anatomical sketches of poor quality. Da Vinci was the only man whose product exhibits great originality and independence. His anatomical work was on the plane of that of Vesalius but his sketches were not printed until long after.

The practice of dissection by medical men was not so actively opposed by the church as is generally supposed. A superficial reading of the bull of Pope Boniface, *de Sepultis*, issued in 1300, has led to the statement that it was directed against the practice of dissecting for scientific purposes, but it was, in reality, a proscription of the practice of dismembering the bodies of dead Crusaders, in order that their bones might be more readily transported home for burial in consecrated ground.

The practice of plagiarism was widespread during this period. Publishers and authors engaged in it in a wholesale way; both sketches and text were commonly copied without credit being

given. The ethics of the rights of intellectual property were unrecognized. The earliest printed sketches were derived from manuscript sources and, these, in turn, were based upon the traditional anatomy, chiefly of Galen and his commentators. Now and then a touch of original observation was added to the traditional figures but they were not perfected. Dependence on authority was still the deep-seated method of the intellectual life, and the rise of independent observation was slow. But, the better intellects were opposing it, and with all these limitations the light of the renaissance was breaking. Dependence on authority was giving way, and, finally, thanks to the work of his predecessors, Vesalius was able to establish a new method based on observation and reason. With the publication of his *Fabrica* in 1543, there was ushered in the era of good illustrations of anatomy. The prevailing mental habit of the time was now at least partly overcome and the era of independent observation was started.

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## MINIMAL SIZE REDUCTION IN PLANARIANS THROUGH SUCCESSIVE REGENERATIONS

S. J. HOLMES

It is a familiar fact that very small pieces of fresh water planarians will regenerate and give rise to minute individuals closely resembling the original form. I have endeavored to ascertain how far reduction of size in *Planaria maculata* may be carried without causing a failure to give rise to a normal individual. With very small pieces of an adult planarian there is a large proportion of cut surface which produces an injurious effect and there are also various mechanical impediments to regeneration; these facts, combined with the specialized condition of much of the tissue, conspire to restrict the regenerative capacity of the parts. In order to eliminate somewhat these factors the device was resorted to of subjecting the animals to a number of successive divisions. A planarian was cut into fifteen or twenty pieces; when these had regenerated into small planarians they were again cut into several pieces. These regenerated into still smaller individuals which in turn were divided, the process being continued until forms were reached which were so small that complete regenerations were no longer obtained. In this way, when the minimal size limit was approached, regeneration became very slow and many of the pieces lived for months without restoration of the missing parts. As a general rule it may be said that the smaller the piece the more slowly the restorative processes take place. In this way the proportion of cut surface was reduced, the tissue kept in a more plastic condition, and the whole process of regeneration made easier.

Eugene Schultz has studied the reduction in size of planarians from starvation. He found that planarians could be reduced in this way to one-tenth or one-twelfth of their original size. A study of the size of cells of various kinds showed that there was

little reduction in size either of the cells or their nuclei as a result of starvation; the diminution in the size of the body was produced mainly by their reduction in number. Various organs suffered unequally in this process. Organs of copulation, sex ducts and vitellaria were among the first to disappear, the eyes degenerated, and there was a marked reduction in the number of parenchyma cells. The muscle cells suffered little decrease and the number of muscle bands remained unaltered; there was little reduction in the nervous system. The male sex cells were among the least altered. Cells of the intestinal epithelium and the outer ectoderm, while reduced in number, were not reduced disproportionately to the body as a whole.

The study of small regenerated planarians was undertaken in order to ascertain how far the various organ systems would suffer on account of reduction in size and how far the results might be parallel with the effects of starvation. Through successive regenerations it is possible to carry the reduction very much farther than can be done by the withdrawal of food. While starvation may reduce the animal to one-tenth or one-twelfth its original size, by the method of successive regenerations it may be reduced to  $\frac{1}{1000}$  or  $\frac{1}{1500}$  its original size. Many of these very minute animals had practically the same form as the adult. Several specimens were sectioned and careful measurements were made of several kinds of cells and compared with measurements of corresponding cells of individuals of ordinary size. Ectoderm cells, parenchyma cells, cells of the intestinal epithelium were of the same size as in the larger worms. The muscle cells, while less in length, were nearly as thick as in the larger worms. The nuclei were also not reduced in size and therefore bore the usual relation to the size of the cells. The gonads, sex ducts, copulatory apparatus and vitellaria could not be found. The muscular system is well developed, the outer layers being present and only a little thinner than in normal individuals. The number of dorsoventral strands in a cross-section is not more than about one-fourth that of larger specimens and they contain fewer fibers. The alimentary canal has but a very few short branches. The cells are not shrunken as occurs in starved individuals and

cross sections of the diverticula appear much as in the larger worms except for the reduction in the number of cells. The size of the brain and the diameter of the nerve cords bear about the same ratio to the rest of the body as in large individuals. In a few cases there was but one eye instead of two and this was not a median one as sometimes occurs in small individuals but was in the position of one of the lateral eyes. The eye is of normal size, in relation to other parts and it has essentially the usual structure, but there is a great reduction of the number of the retinal cells.

The pharynx, which bears about the same relative proportion to the body as in larger planarians, is composed of the same epithelial and muscular layers. The relative proportion of the parenchyma and digestive organs is little altered in the smaller individuals. The relative thickness of the outer epithelium is however much greater, since it is composed of but one layer of cells which have the same size in the large and the small planarians. Pigment cells occur sparsely scattered over the dorsal surface and appear of enormous size in relation to the rest of the body. On the whole, the small individuals are strikingly like the larger ones in general form and the relative proportions of the systems of organs.

Observations were made on the movements and reactions of these minute forms. Methods of locomotion, exploring movements of the head, reactions to light and contact, responses to mechanical stimuli, righting movements, and various other activities, even down to the most delicate details, were carried out in practically the same way as in individuals of normal size. These facts indicate how effectively the functional unity of the organism is maintained notwithstanding the enormous reduction in the number of its cells.

One factor which probably determines the minimal size which may be attained is the fact that the size of the cells cannot be reduced and there must be a certain number of kinds of cells to preserve the physiological unity of the organism. There must be nerve cells, muscle cells, parenchyma cells, epithelium, etc., if the planarian is to be a planarian. The work of the organism, like

that of a factory, can be performed on a large or a small scale, but as there are many functions to be discharged, and as one cell cannot do the work of another, a point naturally has to be reached somewhere when a further reduction of the number of cells brings operations to a standstill. Matters might work out, however, in a different way by effecting a general simplification of structure, such as occurs in the reduction of *Hydra*. This simplification of structure, which has been compared to a reversal of embryonic development, does not proceed in the planarian very far. The loss of sex ducts and associated organs is of doubtful significance, since these parts often atrophy at certain periods in adult individuals. In attempting to carry reduction below a certain size the cut ends of the pieces heal over and there is little further change; the organism does not transform itself into a simple embryonic stage, and we cannot with safety speak of the reversal of developmental processes (if it be really such) beyond perhaps a few retrogressive steps.

# THE GEOTROPISM OF PARAMOECIUM

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FIVE FIGURES

Are there any free-swimming organisms which are oriented to gravity by means of the shape or contents of the body in the same way as the axis of orientation of certain eggs is determined by the difference of specific gravity of the opposite poles? The body of *Paramoecium caudatum* suggests the possibility of its being oriented to gravity by the difference in buoyancy of the two ends. The posterior end is broader and the anterior end indented by a deep groove. Is the body of *Paramoecium* 'stern-heavy,' and if so, does it account for the ordinary negative geotropism of the animal, the anterior end having a tendency normally to point upward? Various explanations have been propounded for the geotropism of *Paramoecium*, such as difference in water pressure at different depths, difference in pressure on the lower and upper surfaces and finally Lyon,<sup>1</sup> ascribes the geotropic response to the internal stimuli of heavy particles, making the body of *Paramoecium* the analogue of the statocyst of higher forms.

To test the writer's hypothesis, it was thought it might be possible to increase the difference in specific gravity of the two ends by making the animals ingest heavy particles. These would at first lie toward the posterior end near the mouth, making the body more 'stern-heavy,' and so possibly increasing the negative geotropic tendency.

<sup>1</sup>Lyon, E. P., 1905. On the theory of geotropism in *Paramoecium*: Amer. Journ. Physiol., vol. 14, pp. 421-432.

## EXPERIMENTS WITH SUBSTANCES OF HIGHER SPECIFIC GRAVITY

The experiments were carried out as follows: The water used was ordinary tap water boiled to drive away gases. A control experiment was in all cases conducted side by side with the treated specimens. Ordinary test tubes were employed for the experiments. 'Iron by alcohol' was used, being ground up fine in an agate mortar. In order that the number of *Paramoecia* might be practically equal in the two test tubes, the water containing the animals was measured into equal portions. Large numbers were used in order to facilitate observation and comparison of aggregations. In the control test tube was placed a quantity of finely ground iron and after this had settled to the bottom, which occurs very quickly, the measured quantity of *Paramoecia* were introduced into the tube. The others were put in the agate mortar with finely divided iron and stirred for a definite time so as to keep the particles in suspension. In some cases the control animals were similarly stirred before placing them in the test tube in order that mechanical agitation should affect the results equally if at all, since this is known to change the reaction, sometimes, to positive.

The amount of iron ingested is readily observed with the microscope, and a short treatment may be sufficient to cause the ingestion of a considerable quantity. They were allowed in different experiments to ingest the iron for intervals varying from fifteen seconds to five minutes before transferring to the test tube. The control animals and the treated ones were placed in the tubes at practically the same time, so that there would be no difference in time interval to allow for in comparing the movements and aggregations of the animals in the two tubes. As a method of recording the observations, which were made with the naked eye and hand lens, the approximate distribution was indicated on a diagram. This could be made sufficiently accurate to indicate any decided difference that might be noted in the regions of greatest aggregation as well as the general distribution in the two tubes.

Many factors influence the movements of *Paramoecia* so that some cultures aggregate quickly at the top in a ring and others remain distributed, and in some cases move downward. The various unknown factors have been disregarded and comparisons made only to determine whether the treated specimens exhibited a stronger negative geotropic tendency than the ones not treated. The placing of iron filings in the control tube was to eliminate any possible chemotactic factor. Finely divided bismuth and nickel were also tried, but iron was found entirely satisfactory for the purpose of the experiment.

One condition of the experiments is that the iron filings after a time become distributed through the endoplasm more evenly, so that any difference in behavior is to be looked for before this change occurs. A second condition is that an excessive amount of iron may overload the animals apparently and cause them to aggregate at the bottom. The *Paramoecia* rid themselves of the iron after the course of a few hours without apparently harmful effects from a small amount.

One more precaution needs to be stated. The whole quantity of iron in the mortar must be removed with the *Paramoecia*, or a possible loss might occur of some individuals whose tendency was to remain on the bottom, or else the iron must be repeatedly rinsed to remove all of them.

The experiments have been repeated so often that certain results appear as typical, and these will be reported just as recorded.

*Experiment 1* Control tube referred to as No. 1; treated individuals as No. 2. Ingestion of iron for five minutes.

Five minutes after the beginning of the experiment the distribution in the two tubes did not noticeably differ.

In ten minutes there was about an equal collection gathered at the bottom of each tube. To observe the size of these aggregations better the tubes were slightly shaken.

Twenty minutes later there was a large collection at the top of No. 2, very few at the top in No. 1. Looking down from above gave the appearance shown in fig. 1a; fig. 1b gives a side view of the same.

In this experiment, on account of the long treatment, a considerable number had apparently taken on an overload of iron, which would account for the aggregation at the bottom of No. 2.

In one hour and twenty minutes nearly all had collected at the bottom in both tubes, but a collection still remained at the top in No. 2, none in No. 1 (fig. 2)

*Experiment 2.* Animals from the same culture, same day. Ingestion of iron for thirty to forty seconds.

Fifteen minutes later, there was an aggregation in the bottom of No. 1, and a few scattered all the way up the tube. In No. 2 there was a slight aggregation at the bottom and a considerable collection above the middle of the tube.

In twenty minutes the collection appeared as shown in fig. 3. The smaller number at the bottom of No. 2 was referable to the fact that less iron had been ingested than in the former experiment. Notwithstanding the normal tendency to go downward, there was a decided tendency among the treated animals to go upward.

*Experiment 3.* This is a sample of those experiments with cultures in which the normal tendency was to go upwards and form a more or less dense ring at the top of the tube. Ingestion of iron for one minute. In ten minutes a much denser ring was formed at the top of No. 2, and this inequality remained (fig. 4). The *Paramoecia* in No. 2 moved more slowly than in No. 1. When the test tubes were corked and inverted, no air being allowed to enter, there was the same collection at the top, but not ring formation.

The interpretations of Experiment 3 might differ. The denser ring at the top of No. 2 might be explained as an entrapping of the animals in this region, resulting from their slower movements, on the principle by which Jennings explains aggregations due to chemical influences. At any rate the fact remains that the treated animals swarmed to the top more quickly and formed a denser ring there than in the control. These experiments are less crucial than those with animals having a normal tendency to go downward.

EFFECTS OF INGESTION OF SUBSTANCES HAVING A LOWER  
SPECIFIC GRAVITY

Various substances were tried, but finally paraffin was selected as best adapted for the purpose. Finely divided paraffin was obtained by melting and cooling in hot water several times till the paraffin was thoroughly washed. Then a small amount was melted in a flask of hot water and shaken and then suddenly cooled under the tap. The fine, suspended particles would soon rise to the top. For the control experiment paraffin in particles too large to be taken in was used.

*Experiment 4.* Both Nos. 1 and 2 were shaken at the same time for several minutes and the tubes then allowed to stand. In No. 2 the animals at the end of fifteen minutes were aggregated densely at the bottom at rest. They remained so, under observation, for several hours. After twelve to twenty-four hours all would be found scattered through the tube. In the control no noticeable effect was produced by the paraffin. The dense aggregation at the bottom of No. 2 was obtained with cultures of the different types (fig. 5).

The inference that the posterior end was buoyed up by the paraffin particles, so as to orient the animals downward, is the conclusion of the writer, for the present at least. \*

## DISCUSSION OF RESULTS

The above experiments seem to place the gravity orientations of *Paramecium* on the same plane as the orientation of the axis of certain eggs by specific gravity.

Lyon compares *Paramecium* to a statocyst. It is not easy to see how an animal revolving continually on its axis could react to the localization of an internal stimulus. While such a stimulus might conceivably act effectively in an antero-posterior direction, the explanation offered in this paper seems simpler, at least to the writer.

Lyon centrifugated *Paramecia* strongly into a tube ending in a capillary bore and found that the animals moved with the anterior end outward, and that certain heavier particles were driven into the anterior end.

The inference that the anterior end is heavier is contrary to what the shape of the body would indicate, unless the heavier particles are located anteriorly. The writer wishes to suggest as an explanation of Lyon's experiment that in strong centrifugation the same effect is produced at the outset as by mechanical agitation, i.e., the reaction changes to positive. Jensen<sup>2</sup> showed that with weak centrifugation the animals moved centripetally.

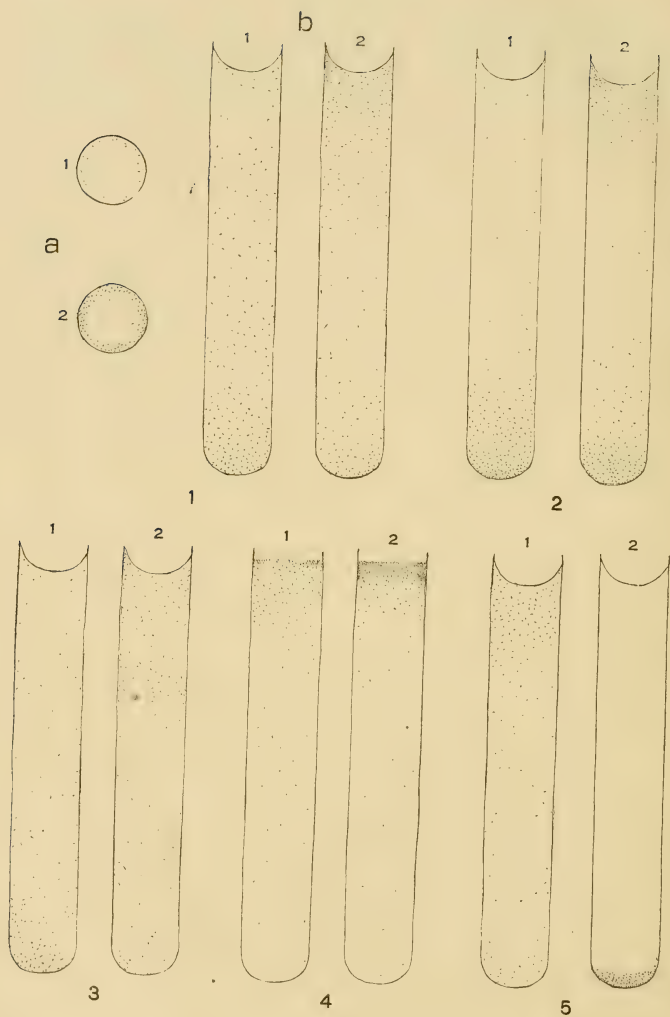
It is conceivable that the pull of gravity on the heavier posterior end may produce a tipping effect which is able to orient passively but is too weak to stimulate. When, however, the centrifugal effect exceeds the pull of gravity and produces too sudden an orienting tendency, this may act as a stimulus to the animal to resist as in the ordinary rheotropic reaction against the current. When strongly centrifuged the animal takes a position so that it moves in the water just as the water moves past it in the rheotropic response. In other words, if it allowed itself to be oriented by the centrifugal force with the posterior end in advance, its relation to the water would be the reverse of what it is in the rheotropic response. The writer repeated Lyon's centrifugation experiments and the explanation here given, namely, that the animal is able to react at the outset of centrifugation, seemed to him the most satisfactory explanation of the fact that all are found to move with the anterior end outward.

So also in shaking, on account of the difference in buoyancy of the two ends, the heavier end will move more rapidly, and this may become effective as a stimulus, causing the movement downward.

If the explanation here given hold, we have in the normal, quiet, geotropic reactions of *Paramecium* an example of a purely mechanical tropism. The term tropism would be here applied to a passive orientation not involving the irritability. If it be desirable to use the term 'tropism' for such a kind of orientation,

<sup>2</sup>Jensen, P. Ueber den Geotropismus niederer Organismen; Arch. f. d. ges. Physiol., Bd. 53, pp. 428-480.

it is evident that it forms a separate class from those that involve a change of irritability. When, however, a stronger force is substituted for gravity so as to produce a sudden orientation, the irritability is affected, and the animal reacts to the change.



# THE FORMATION OF THE SPERMATOPHORE IN ARENICOLA AND A THEORY OF THE ALTER- NATION OF GENERATIONS IN ANIMALS

ELLIOT ROWLAND DOWNING \*

FOUR PLATES AND SEVEN TEXT FIGURES

## CONTENTS

Location of the gonads.....	1002
Methods.....	1002
Limits of the gonads.....	1004
General statement of spermatophore formation.....	1006
Breeding habits.....	1007
Degeneration and phagocytosis of the gonad.....	1008
Extra blood vessels for aeration.....	1009
Origin of the spermatogonia from peritoneal cells.....	1011
Structure of the gonad.....	1012
The spermatophores, their formation.....	1013
Their discharge into the body fluid.....	1014
Their analogy to blastulae and gastrulae.....	1015
Their behavior when ripe.....	1016
The giant spermatogonia.....	1017
Spermatogonial macromeres and micromeres.....	1018
The spermatophore originates from one primary spermatogonium by a process of cleavage and invagination.....	1021
The spermatophore an individual—the gametozoon.....	1022
The alternation of generations.....	1023
Botanic use of the term.....	1023
Usual zoological significance.....	1023
Alternation and chromatin reduction.....	1023
Independent phenomena.....	1024
Chamberlain's theory of the alternation of generations in animals.....	1028
Objection to it.....	1029
Limits of the gameto- and sporo-generations.....	1029
Illustrated by graphic life histories.....	1030
Sexuality and reduction.....	1033
Adjacent phenomena.....	1033
Not necessarily causally related.....	1033
The primitive animal type and the development of the alternation of genera- tions as found in Arenicola.....	1034

The gametozoon a 2x form.....	1037
The common plant and animal prototype.....	1037
The primitive position of the reduction phenomenon.....	1038
Its shift toward typical plant and animal positions.....	1038
Reduction and tetrad formation.....	1039
Beard's hypothesis of alternation of generations in animals.....	1039
Bibliography.....	1041

#### LOCATION OF THE GONADS

The gonads of the Arenicolidae are located on blood vessels which run diagonally across the surface of the nephridia.

The typical somites of these worms are composed of five annuli, one of which bears the setae. Three annuli are anterior and one posterior to this setigerous one. There are six pairs of nephridia in *Arenicola cristata*, situated in the fifth to the tenth somites inclusive, a pair for each somite. Each nephridium consists of a funnel, body and bladder. The funnel has a sagittate dorsal lip set with ciliated plates and a lobed convex ventral lip; between the lips is the nephrostome. The body is club-shaped and connects, near its larger anterior end, with the funnel, at its posterior end with the bladder. The bladder is roughly spherical and opens to the exterior by a narrow tube through the nephridiopore.

Each funnel is attached by the outside of the dorsal lip to the ventral surface of an oblique muscle, at some little distance from the attachment of the muscle to the body wall; so that the apex of the arrow-shaped funnel points downward and forward. The body of the nephridium passes up, back, and outward from its juncture with the funnel, to the bladder, which lies against the body wall close to the line of insertion of the oblique muscles to the sides.

#### METHODS

The ordinary method of pinning the animal out for dissection so pulls the nephridia that the shape, particularly of the delicate funnel, is distorted. The method followed has, therefore, been (1) to stupefy with 70 per cent alcohol, adding it rapidly, drop by drop, to just enough sea water, in a long dish, to cover the animal. Stupefaction, with complete relaxation of the powerful muscles of the body wall ensues, in *A. cristata*, in from ten to thirty

minutes; in *A. clapedii*, in from three to eight minutes. (2) With a hypodermic syringe, sufficient preserving fluid is injected to distend the body; the whole worm is then immersed in the preservative. After hardening, the nephridia, when dissected out, have the shape and relations described above.

Details of the methods of preservation will be reserved for a later paper in which the chromatin changes and other histological matters will receive attention. It will be sufficient now to state that testes preserved in strong Flemming, Bouin and vom Rath fixing fluids have given best results. The forming spermatophores have been studied in fresh body fluid, stained with methylen blue or neutral red; or in smear preparations killed by exposure to osmic acid fumes and fixed in Merkel or in vom Rath; or from sections prepared by squirting body fluid, freshly drawn, immediately into hot corrosive-acetic or Flemming, then hardening in alcohol in the usual way and sectioning in paraffin. Warm iron haematoxylin and Bordeaux red or saureviolett and fuchsin have been among the most successful stains.

As the author has elsewhere published a description of the relations of the blood vessels to the nephridia in the Arenicolidae, the following brief description will suffice here. Each nephridium of *A. cristata* is supplied with blood by a branch of the ventral blood vessel. This afferent vessel, on approaching the nephridium, branches to the setal sac and gill (if present), to the integumentary vessels, notably the dorsal-longitudinal, and to the nephridium. The branch to the nephridium enters the anterior angle of the sagittate funnel and after traversing it, passes on to the upper surface of the body of the nephridium, which it crosses diagonally from the anterior inner to the posterior outer side. Peripherad to the nephridium, it joins the nephridial longitudinal vessel. From the point of emergence from the funnel on to the body of the nephridium to its juncture with the nephridial longitudinal vessel, the blood vessel is designated the gonadal vessel, since upon it the gonad is found. Gonadal tissue is also found, to a slight extent, upon the nephridial longitudinal just anterior to its attachment to the gonadal vessel (plate figs. 1-6). These figures show the location and relative size of the gonads in the several species.

They are taken from typical nephridia and are drawn to the same scale. In each case the individual selected was an average sized worm and was taken at a time of the year, too, when the particular species was approaching the maximum of its breeding activity, so the gonad should have its maximum size. The extent of the gonad evidently varies greatly. It is confined to a relatively small area on the gonadial vessel in *A. grubii* and *claparedii*. It is much more extensive in *A. cristata* and about equally so in *A. marina*. The gonad achieves its greatest size in *A. ecaudata*. The relatively immense testes of this species are due to the fact that the sperm are retained within them until nearly mature while in the other species the spermatogonia are early discharged into the body fluid, there to undergo the major part of their development. The large bladders of the nephridia of *A. grubii* seem similarly due to the fact that they are used as storage rooms for the sexual products after their formation, while in *A. cristata*, *marina* and *claparedii* these are held in the general body cavity.

#### LIMITS OF THE GONADS

The gonad usually surrounds the blood vessel, It appears as a light yellow incrustation, varying in thickness with the season. As it decreases in size it occupies a more and more restricted area on the posterior portion of the gonadial vessel. Not infrequently the other blood vessels adjacent to the nephridia, the dorsal longitudinal, the nephridial longitudinal and the nephridial branch of the afferent, are covered with a similarly appearing incrustation, but on microscopic examination, the material is found to be chlorogonous, never gonadial. Furthermore, the gonad is confined to the blood vessels of the second to the fifth nephridia, inclusive, in *A. cristata*. Over a hundred males have been carefully examined: in 6.2 per cent, one or both of the first nephridia had the gonadial vessel slightly to plainly coated with the yellowish incrustation; 11.3 per cent of them had the gonadial vessel of the sixth nephridium coated. Such nephridia have in all cases been removed and sectioned and with one possible exception, the sections show the incrustation to be chlorogonous. In a single instance the gonadial vessel of a sixth nephridium had upon it a few cells looking

like degenerating spermatogonia. As these worms were selected at intervals throughout the year so they would be representative, we may safely conclude that the vessels of nephridia one and six never bear active gonads, while there is slight evidence that the gonadial vessel of the sixth nephridium bears a degenerating gonad.

The limits of the gonads have not been as carefully studied in the other species of the Arenicolidae as in *A. cristata*. Yet I have examined, macroscopically, several dozen specimens of each of the other species, *claparedii*, *ecaudata*, *grubii* and *marina* and have examined microscopically the blood vessels when any doubt could exist, with results confirmatory of the statements of previous authors, notably Gamble and Ashworth, as follows: *A. ecaudata* has thirteen pairs of nephridia in setigerous segments 5-17, *A. marina* six, in segments 4-9, *A. grubii* and *A. claparedii* each five pairs in segments 5-9.

Presumably the Arenicolidae have evolved from a more generalized polychaete in which nephridia and gonads were segmentally repeated organs. Both organs have gradually been confined to a smaller and smaller region. This gradual reduction seems to be well illustrated within the group as indicated by the number and position of the nephridia given above. Moreover, according to Gamble and Ashworth, the first pair of nephridia of *A. marina* are frequently absent and the last pair occasionally. Lillie remarks of the nephridia of *A. cristata* that "The two earliest formed pronephridia, those of somites IV and V, degenerate at a comparatively early period in the development. The remaining six pairs (in somites VI-XI, inclusive) are directly transformed into the definitive adult nephridia." Fauvel ('99) states that the number of nephridia in *A. ecaudata* is only occasionally thirteen; that twelve is the usual number, the last pair of nephridia being absent from his specimens. In the two hundred and more specimens of *A. cristata* examined I have found only three cases of variation in the number of nephridia. In two of these the first pair of nephridia was wanting; in the third only the funnel was present in the sixth right. I have yet to find variation in the other species.

The gonads are more restricted than the nephridia. As noted above, the first gonadial vessel in *A. cristata* never bears gonads, the sixth rarely and then degenerating cells only. In the other species the first nephridium never has a gonad. Here then is a case in which *the genital cells show an evolutionary character, the tendency to restriction, more emphatically than the somatic cells.*

#### GENERAL STATEMENT

A section through the testis of any of the Arenicolidae shows, ordinarily, a mass of cells of two or three sizes (fig. 7-9): These are the spermatogonia of successive generations. The larger ones lie adjacent to the blood vessel. At the periphery of the gonad spherical bunches of spermatogonia or occasionally single ones are seen to be loosening from the general mass preparatory to discharge into the body fluid (except in *A. claparedii*). In this fluid the further divisions of the cells result in the formation of hollow spheres of spermatogonia (fig. 10), the last generation of which grow to spermatocytes. These, by the customary two divisions, become the spermatids (fig. 11) the cells still adherent in the spherical masses, which are meanwhile however altering their shape and becoming saucer-shaped, in *A. cristata* (fig. 12) slightly biconvex in the other species, except in *A. claparedii*, in which the successive divisions occur in the testis and the sperm are discharged into the body fluid. These sperm masses are the spermatophores, (fig. 13). The body fluid of *A. cristata* is loaded with these spermatophores (or with the eggs) except for a short time just after the discharge of the sex products, and in the other species, except *claparedii*, for weeks before the eggs are deposited. There are present, of course, other elements, body cells, chloragogue cells, etc., but the dominant objects are the eggs and the spermatophores. Finally the tails of the sperm are stiff and are aggregated into conical masses (fig. 12).

Toward the close of the breeding season, I have found, both in *A. cristata* and *A. claparedii*, exceptionally large spermatogonia discharged singly from the gonad, in addition to the customary masses described above. These develop in a somewhat different and highly instructive manner, as will be described later.

## BREEDING HABITS

The breeding season at Woods Hole lasts, for *A. cristata*, from about the first of May until the end of August, attaining its maximum during June. The cylindrical jelly strings containing the eggs are found in the shallow water over the littoral mudflats at low tide, one end attached at the burrow of the worm. The string lies on the bottom almost afloat. From field and laboratory observations I conclude that the male lies adjacent to the female during the discharge of the eggs and simultaneously discharges the sperm through the nephridiopores. The following facts support the statement: (1) I have repeatedly captured both male and female at an egg string when the latter was just beginning to appear. That one frequently fails to find both animals is, I presume, due to the fact that they burrow with extreme rapidity. If the tail of an animal be exposed with one stroke of the digger it often disappears before the next stroke can be taken and only very hurried work makes capture possible. When two worms are at the same burrow the chances of getting both are not great. It is to be remembered also that it is necessary to capture the worms in order to determine the sex as there are no external differences. To determine the sex without killing the animals I have examined a small drop of the body fluid withdrawn from the body cavity by means of a hypodermic syringe. The presence of either eggs or sperm can usually be determined by the naked eye. (2) The discharge of eggs and sperm has been seen to occur through the nephridiopores in worms kept in pans in the laboratory. (3) I have been reasonably sure that male and female were coöperating in the formation of the egg string in animals kept in aquaria in the laboratory. At best, however, the details of the process are obscure, since the animals, even when close to the glass, are pretty well covered with sand.

The conspicuous egg strings make *A. cristata* the easiest species to locate when depositing the eggs. The other species probably lay the eggs in the sand and débris among which they live. The times of their sexual maturity are fairly well established. *A. cristata* is found to mature at about the same time at Naples as at

Woods Hole, i.e., June to August (LoBianco). *A. marina* is captured with mature eggs and sperm at Woods Hole in the early spring, April and May. It is found mature on the English coast in the spring (the laminarian variety) and summer (the littoral variety) according to Gamble and Ashworth. My specimens of sexually mature *A. ecaudata* were taken at Plymouth in April as also were specimens of *A. grubii*. The latter and *A. clapedii* I found mature at Naples in May. Both of them have been found breeding much earlier there (LoBianco), *A. clapedii* beginning as early as November and continuing throughout the winter and spring. By using a haemocytometer I have estimated the number of spermatophores per cc. of body fluid in a mature male at about forty million. Each spermatophore will average in the neighborhood of a thousand sperm. A good sized male *A. cristata* will easily contain twenty-five or more cc. of body fluid, that is, a quadrillion sperm, ready to be discharged when fertilization is to be accomplished.

#### DEGENERATION AND PHAGOCYTOSIS

At the close of the breeding season the body cavity of the male contains very little sperm. In only 10 per cent of the September specimens of *A. cristata* taken at Woods Hole was sperm present in any quantity. In 60 per cent so little was present that it was impossible to determine the sex except by sectioning the gonads. During August and September the gonad is at its minimum size. This I have determined in two ways: First, by macroscopic examination. The gonad is apparent on the blood vessel even to the naked eye. Examination of the animals throughout the year, with record of cases in which the gonad is plainly evident on nephridia two to five, shows that in August and September the gonads show least frequently. They become plainer during the fall, achieve the maximum size from December to March and then gradually become smaller again. Second, the same results have been reached by making serial sections of nephridia for each month and making camera lucida drawings of the largest cross section of the gonad as compared with the blood vessel on which it lies. The third and fourth nephridia are used preferably for the com-

parisons as they show the maximum gonad development. In September the blood vessel shows only a thin line of gonadial material in a very limited area. This grows rapidly from month to month until the gonad is of large size and is giving off spermatophores into the body cavity. Sections from the December and January worms show the maximum relative cross section of the gonad. It gradually decreases as its substance is given off into the body fluid as forming spermatophores during spring and early summer. During June the body cavity has its maximum of sperm. By the last of July and in August fibrous degeneration is evidently going on in the gonad (fig. 8-9), and the disintegrating remnants of gonadial tissue are being ingested by abundant phagocytes (fig. 14).

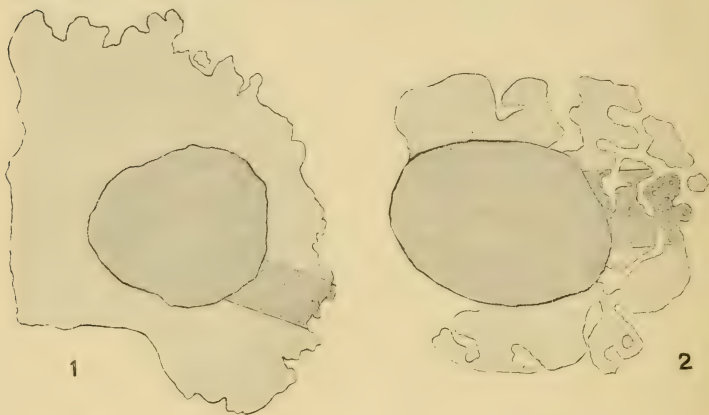
Though specimens of the other species have not been collected in such quantity as *A. cristata* throughout the year, yet enough of each has been seen to make quite certain that the description given will equally well apply to them, making allowance for the changed breeding period, always excepting *A. ecaudata*.

By October the degenerative changes have ceased in *A. cristata* and multiplication of the gonad cells has begun again. A month before the gonad attains its maximum size the peripheral cells of the gonad are beginning to break away in small masses and float in the body fluid. The later development of these spermatophores goes on in the body fluid. In October the margin of the gonad appears entire in section; in February and later the margin appears very ragged as the masses of gonad material are constantly discharging (compare figs. 7 and 8 and text figs. 1 and 2).

#### EXTRA BLOOD VESSELS

It will be seen then that the maximum size of the gonad does not coincide with the height of the breeding season. This is marked rather by the greatest abundance of the mature spermatophores in the body fluid. In September the body fluid contains few or no sperm. By October they are appearing and steadily increase so that by November or December the fluid is crowded with the masses of developing sperm. An interesting development of the circulatory system goes on simultaneously with this

accumulation of growing spermatophores. During December the diagonal muscles in the region of the first nephridium especially and to some extent in the second are apparently becoming 'hairy.' This appearance is due to the numerous long, fine blood-vessels attached at one end to a large vessel, the other floating freely in the body fluid. Similar vessels have been noted before



Text fig. 1. Outline of the testis of *A. cristata* in October. The gonadal material lies around the blood vessel. The dotted area corresponds to that shown in detail in fig. 7, pl. 2.

Text fig. 2. Outline of the testis of *A. cristata* in February. The dotted area corresponds to that shown in fig. 8, pl. 2.

in *A. clapedii* and *A. grubii* by Gamble and Ashworth (p. 518) with no suggestion as to their probable function. They seem evidently a device for the better aeration of the body fluid and the elimination of its wastes while it is heavily loaded with the developing spermatophores. They disappear in *A. cristata* in early summer when the sperm are fully formed.

## ORIGIN OF THE SPERMATOGONIA

Lillie says of *A. cristata*:

The early germ cells in connection with each nephridium become distinguishable soon after the appearance of the blood vessel of the latter and arise as a proliferation of the peritoneal cells of its walls. They appear first on the anterior and first formed portion of the vessel, i.e., in the region immediately adjoining the posterior angle of the funnel. The germ cells usually appear on their respective nephridia in the order of formation of these organs, i.e., in order from before back.

This peritoneum out of which the germ cells differentiate is derived from large teloblastic nuclei located at the posterior portion of the embryo in the growing zone, which nuclei Lillie thinks are the homologues of the definite teloblast cells found in such forms as *Clepsine* and *Lumbricus*. They in turn are apparently direct descendants of 4d, one of the fourth quartette of blastomeres derived from the macromeres at the sixth cleavage, as described by Child.

I have endeavored, in *Arenicola*, to discover some constant characters in the germ cells, which, appearing also in certain of the peritoneal cells, would enable me to trace the germ cells back through successive generations to the derivatives of 4d. But in this I have had no success and we must conclude that, as far as optical characters are concerned, the germ cells are indistinguishable by present methods from the other peritoneal cells. In other words, the differentiation of the germ cells is probably called forth by stimulation of adjacent cells due to progressive inherent changes.

Such a differentiation of the germ cells from the peritoneum is common in the annelida. "Die Bildungstätten der Geschlechtsproducte gehören bei den Ringwürmern genetisch den epithelialen Wandungen des Cöloms an und erscheinen in Folge diesen als directe Abkommlinge der Mesodermstreifen oder des secundären Mesoderms." (E. Meyer, Studien über den Körperbau der Anneliden, III.)

Gamble and Ashworth state ('00, p. 31) in regard to *A. marina* that "In large *Arenicola*, at certain seasons, the vascular process has no gonad and it is possible, as Cuenot ('91) suggests, that a formation of the amoeboid corpuscles of the coelom takes place at this point when the animal is not breeding." If this be true, evidently the gonad cells must form anew, as a proliferation of the peritoneal cells in adult life as well as in the embryological development and the distinction of germ cells and soma would be hypothetical. If so, too, the annual appearance of the germ cells must be due to a cyclical change in the organism contemporaneous with or due to the seasonal change without. Of course this must be true of their rapid increase anyway.

I have examined many specimens of *A. cristata* taken in September in which the body cavity showed no sperm, sectioning nephridia on whose blood vessel naked eye examination showed no gonad, but have always found under the microscope some gonadial tissue. So that I feel reasonably certain that in this species at least, after once the primitive spermatogonia appear in the embryological development, they do not disappear.

#### STRUCTURE OF THE GONAD

These first few germ cells, then, formed from the peritoneal cells, multiply with rapidity until the entire gonadial vessel is covered with a thick incrustation of them. Division of the peripheral cells now becomes more rapid, so rapid in fact that the daughter cells do not grow to the size of the parent cells before division again ensues. There thus result zones of cells diminishing in size to the periphery of the gonad where they are being discharged into the body fluid. The largest cells, those adjacent to the blood vessel, are about  $12\mu$  in diameter in *A. cristata* ( $11.67\mu$  the average of over one hundred cells). The next smaller size are  $9.36\mu$  in diameter, then  $7.55\mu$  and the outermost  $6.02\mu$ . In *A. claparedii* and *A. grubii* the cells adjacent to the blood vessel are smaller, only about  $10\mu$  in diameter.

Such an ideal arrangement of the cells is never found throughout the gonad. At some points the largest sized cells will at times

delay division until well out toward the periphery. The intermediate sizes may be scattered, with no apparent order, throughout the organ. Not infrequently, cells of the third or even second generation reach the edge and are broken from the mass to float in the body fluid separately or in larger or smaller groups. But frequently the typical arrangement described will hold for large regions of the gonad. The peripheral cells tend to cohere into roughly spherical masses of from ten to fifty or so cells all in the same stage of division and these break away into the body cavity. But smaller masses of cells may be detached, even single cells. In the body fluid division continues rapidly as will be described shortly.

#### THE SPERMATOPHORE

##### *Formation*

The largest spermatogonia, immediate descendants of the peritoneal cells, may be called the primary spermatogonia. During the year, except just before the height of the breeding season, these primary spermatogonia multiply, and after division, the daughter cells grow to the size of the parent cells except toward the periphery of the gonad. In the late fall and winter, in *A. cristata* collected at Woods Hole, the gonad is largely made of the primary spermatogonia; it presents quite a solid appearance. In the spring, however, division of these cells ensues so rapidly throughout the gonad, as it always does at the periphery, that the secondary spermatogonia do not have time to become as large as the primary ones before they divide in turn. Now the division of the cells derivative from a single primary spermatogonium, after it starts on its course of rapid subdivision, seems always to be roughly synchronous. The gonad taken during the fall and winter presents a somewhat mottled appearance along the margin when sectioned and stained, due to the prominence of these masses of cells in division among the relatively inactive primary spermatogonia. In the spring this mottled appearance pervades the whole gonad, for here and there a primary spermatogonium will start on its course of rapid subdivision, giving rise to two, then four, eight, etc., cells, which at first lie close together

as a solid mass, but later have a cavity at the center, the segmentation cavity. These forming spermatophores, for such they are, move to the periphery in order to break away into the coelomic fluid. So in spring and early summer the gonads have, when sectioned, a very ragged appearance. Deep bays and systems of lacunae run into the mass so that the spermatophores may be discharging not only at the periphery but are moving out from many spots in its interior (figs. 7 and 8).

There is frequently protoplasmic material at the center of this early spermatophore. It is derived from the disintegration of cells which, while the spermatophore is forming in the body of the gonad, come to assume a central position. Degeneration goes on as the cells move toward the margin of the gonad so that by the time the margin is reached little is left of the central cells. Occasionally strands of protoplasm run from some of the cells to a central point (fig. 15), suggesting a figure such as Calkins has shown for *Lumbricus*, as if the cell walls formed peripherally before they do centrally. This is an exceptional condition, however, and I am confident that no such interpretation is to be put upon it in *Arenicola*. Figure 16 is much more usual. In the more mature spermatophores one can, by carefully tapping the cover glass, cause the spermatogonia to break away from the central portion, leaving it as a colorless sphere containing occasionally a few granules. This does not ordinarily stain, has slight consistency and seems like a drop of slightly viscid fluid. Not infrequently even this disappears and the spermatogonia break away without leaving any residual mass. The central cells are then absorbed very early and the central remnant is possibly excretory in its nature, the products of anabolism that have diffused into the cavity.

#### *Discharge of the forming spermatophore*

The cells are customarily discharged, as has been stated, from the surface of the testis into the body cavity in roughly spherical masses. The constituents measure about  $6\mu$  in diameter in *A. cristata*. Three divisions at least follow and probably more,

since the cells are growing at the same time they are dividing in the nutritive body fluid. Finally, the last generation of spermatogonia is formed, measuring about  $2.9\mu$ . They transform into the spermatocytes of the first order, with a diameter of  $3.2\mu$ . These divide to form the spermatids with a diameter of about  $2\mu$ . In *A. clapedii* and *A. grubii* the spermatids are somewhat larger, measuring some  $2.4\mu$  in diameter; in the other species the cells vary very little in size from the measurements given for *A. cristata*.

### *Analogy to blastulae*

There is thus formed a hollow sphere of cells, reminding one of the blastula of the segmenting egg (fig. 23). This likeness is even more emphatic when one follows the history of a single spermatogonium when freed from the surface of the testis. It will be recalled that as well as the spherical masses of several dozen cells, smaller masses, even individual cells, are discharged into the coelomic fluid, so that one finds in this fluid all stages in the formation of the spermatophore from the one-celled condition up to the completed structure. Naturally the resulting spermatophores vary very decidedly in size according as they have origin in a single discharged spermatogonium or in a coherent mass of such cells. Measurement of the mature sperm masses gives a variation, their diameters ranging from one to eight.

The single spermatogonium, floating freely in the coelomic fluid, divides into two, then four, eight, sixteen cells, etc., simulating in general appearance the cleavage of an egg. Further discussion of this matter will be taken up below when the division of certain giant spermatogonia is considered (figs. 17-25).

During the process of cell multiplication the forming spermatophore is constantly increasing in diameter. Since the cells form only a single layer, the smaller they become the thinner the wall of the sphere is and hence the larger it may be with a given amount of protoplasmic material. This protoplasmic material must also increase in amount during the spermatogonial divisions by appropriation of nutritive material from the coelomic fluid, for the spheres increase in diameter more than the mere thinning of their

walls would account for. This is not true for the later stages, however, for with the formation of the spermatids a decrease in the size of the individual cells accompanies the transformation of the spermatids to the spermatozoa.

Contemporaneously with the change of the spermatids to spermatozoa, or even beginning when the cells are yet spermatocytes of the second order, a change in the shape of the mass of cells occurs. In *A. cristata* the spherical mass invaginates in a manner that forcibly suggests the invagination of some egg blastulae to form the gastrulae (fig. 24). The gastrula-like mass remains cup-shaped, the mouth wide open, the lips never approximating to suggest a closure of the blastopore. Usually before invagination is complete, the cup begins to flatten out, becoming saucer-shaped, which is the form of the mature spermatophore in this species (fig. 25).

This phenomenon is apparently merely analogous to the process of gastrulation in the egg. Presumably the physical relations between the cell mass and the surrounding medium happen to be such that invagination ensues with regularity in this one species. Body fluid freshly drawn by means of a hypodermic syringe shows these gastrula-like forms, as do also preparations made by fixing the coelomic fluid in a variety of fluids. In other species no such thing happens, but the spherical mass of cells merely flattens out to form a biconvex spermatophore. There is however in the spermatogenesis of this group a phenomenon which is really homologous to the segmentation and gastrulation of the egg and which will be considered in the discussion of the giant spermatogonia.

### *The ripe spermatophore*

As the spermatids transform into spermatozoa the cells elongate, their long axes at right angles to the surface of the saucer-shaped or biconvex mass (fig. 25). The nuclei stain with increasing intensity. The tails of the sperm appear as stiff rods, finely attenuate, held rigidly at right angles to the head and gathered

together into one or several bundles (fig. 12). The head of the sperm is about  $2\mu$  in length,  $1.22\mu$  in transverse diameter, while the tail is ten to twelve times as long as the head.

When the coelomic fluid is drawn by means of a hypodermic syringe and placed in sea water, if the animal is not a perfectly 'ripe' male, the spermatophores remain intact, the rigid tails perhaps moving slightly but stiffly. If, however, the sperm masses be quite mature, those in this condition will show movements, the immature ones remaining quiescent. The tails move at first stiffly through varying arcs, the point of attachment to the head as the center. The bundles of tails disentangle and all the tails come to lie at right angles to the surface of the spermatophore. Now vigorous movements of the tails ensue, stiffly at first; then the tails become supple and undulatory movements begin. The spermatophore now disintegrates and the sperm swim away. This process is much more rapid if eggs be also added to the sea water and more rapid still if some of the slime from the surface of the body of the female be put into the water.

#### THE GIANT SPERMATOGONIA

The fact has before been mentioned that toward the height of the breeding season the margin of the gonad bears exceptionally large spermatogonia which are discharged singly into the body cavity. The primary spermatogonia in *A. cristata* are usually about 11 to  $12\mu$  in diameter. But these giant spermatogonia achieve a diameter of some  $17\mu$  before they are freed from the surface of the gonad to undergo their farther development in the coelomic fluid. During July and August they appear in the gonads of *A. cristata* at Woods Hole. Similar cells were found in the gonads of *A. clapedii* collected at Naples the last of May and in *A. grubii* taken at Plymouth in August. At this time the margin of the gonad is very ragged, and fibrous degeneration with phagocytosis is going on in the parts adjacent to the blood vessels (fig. 8).

## SPERMATOGONIAL MACROMERES

The giant spermatogonium contains a very large nucleus and a prominent nucleolus (fig. 17). When shed into the body fluid the cell undergoes an interesting development. It divides unequally, producing one large and one small cell, (fig. 18). The small cell next divides, giving a three-cell stage. Unequal division of the large cell then occurs, producing a four-cell stage seen in polar view in fig. 19. It is so evident that these early stages in the division of the giant spermatogonia are at least roughly similar to the cleavage stages of the egg in *Arenicola* that we may modify the nomenclature of the latter to describe clearly the former. Just the order of cleavage of the four cells I have been unable to determine except that the three small cells divide before the large one, differing in this respect from the division of the egg blastomeres. When all have divided we have an eight-cell stage consisting of four large cells, the spermatogonial macromeres, and four small cells, the spermatogonial micromeres. The position of the cells (figs. 20, 21), shows that the third division has evidently been a dextrotropic one as it is in the egg cleavage. Up to the sixteen-cell stage it is reasonably certain (and I think for at least one additional cleavage) that the cell lineage of these giant spermatogonia is homologous to that of the egg. Spermatogonial blastulae and gastrulae form much as in egg development. The figure of the sixteen-cell stage, (fig. 22) as indeed all these figures of the cleavage of the giant spermatogonia, are camera lucida drawings done with exceptional care, under a one-sixth inch objective and a one-half inch ocular at the level of the table. It is needless to multiply sketches as they would simply be duplicates of the admirable figures already given by Child for the cleavage stages of the egg.

Shortly after the sixteen-cell stage the macromeres disappear from the surface and migrate into the segmentation cavity. If one slightly crush the spermatophores in the body fluid under a cover glass, the great majority will show the blastophore exuding from the center of the spherical masses, yet a few will show four to six rather large cells which escape from the cavity. This is

especially true at the height of the breeding season. Spermatophores containing such cells are produced. I take it, from the spermatogonia that are liberated singly from the gonad, notably the giant spermatogonia. The large cells are the macromeres and possibly some of the first quartette—in other words, the homologues of the mesentomeres. The spermatophores consisting of a hundred or so cells never show such differences, all giving under pressure, the same so-called blastophore, apparently a drop of fluid, perhaps enclosed in a very delicate sheath, the fluid staining fairly deeply with methylen blue but scarcely at all with neutral red or other stains tried. It seems reasonably certain therefore that the invaginated mesentomeres disintegrate promptly to form the nutrition for the developing spermatophore.

It is manifestly difficult to determine with exactness the order of cleavage and the relations of the cells. These enlarged spermatogonia occur only at the height of the breeding season and then make up a very small per cent of the developing spermatophores in the body fluid since these have been accumulated by the ordinary method for months. One must determine what occurs by the chance finding of successive stages, a laborious process, since the percentage of the desired material is so small. Presumably the cleavage of the giant spermatogonia might be watched if one could keep the body fluid under normal conditions. But it coagulates in the course of a few minutes after removal, the body-fluid cells cohere in masses and all other cells, too, cease their activity. When stages are found in the development of these giant spermatogonia it is not easy to determine the exact relations of the cells, for the cell mass is small and transparent, even if stained; furthermore it is difficult to manipulate the cell mass without breaking it as it is only about one four-hundredth the size of the developing egg. Still I am confident of the above statements, as I have worked with the living material, stained whole mounts and sections. The results stated have been repeatedly confirmed during several summers at Woods Hole, working on *A. cristata* and have also been confirmed with the living and fixed material of *A. claparedii* and *A. grübii*.

After my attention had been caught by the peculiar egg-like cleavage of these giant spermatogonia which float freely in the body fluid, I hunted carefully for the early developmental stages of the normal sized spermatogonia that are occasionally set free singly in the coelom. So far as I can find, their development is the same as that just described for the giant form.

One other interpretation might be suggested for these giant cells, namely, that they are tiny eggs which cleave in the body fluid to a certain point and then disintegrate. It is well known, of course, that such hermaphroditism of the gonad occurs when degenerative changes are going on in it. But such an explanation seems negatived in this case by the following considerations—

1. If they are developing eggs they would be undergoing cleavage long before they reach normal size, in fact when only about one four-hundredth of the size of the egg when it is normally ready to be fertilized and begin cleavage.

2. The normal sized spermatogonia undergo a similar cleavage when they are liberated singly in the body fluid.

3. At the height of the breeding season, when these so-called giant spermatogonia are present, there are also found quite frequently giant spermatozoa whose volume bears about the same relation to the volume of the normal sized sperm as the volume of the giant spermatogonia bears to the volume of the usual primary spermatogonia.

It is to be noted that these exceptionally large spermatogonia appear toward the close of the breeding season. It is at this time that the body fluid is supercharged with spermatophores, evidently taxing the respiratory and excretory organs to the limit of their capacity. For it is at this time that the blood vessels develop in numbers like a thick growth of hair on the first and second nephridia and the adjacent muscles in *A. cristata* and in similar positions in the other species. At this time, too, the gonad is invaded by phagocytes, while the portions adjacent to the blood vessel suffer fibrous degeneration. It seems quite likely then, since the respiratory organs are taxed to the utmost, that an oxygen starvation sets in in the gonadal tissue, inducing fibrous

degeneration and phagocytosis. The growth of the giant spermatogonia may be due to the same general causes, the accumulation of wastes so changing the osmotic relations between cell content and the surrounding medium that an increase in size results, either from accumulation of materials customarily excreted or through increased absorption from the nutritive fluids that bathe the cell.

#### THE SPERMATOPHORE DEVELOPED FROM ONE SPERMATOGONIUM

In the light of these facts regarding the development of the giant spermatogonia and those of normal size that float freely in the body fluid, it is now worth while to review the formation of the ordinary spermatophores—those that are developing from the cells in the body of the gonad. A primary spermatogonium divides into two, four, eight cells, etc. Some of these cells move to the center of the mass and disintegrate to form the blastophore whose substance is absorbed as nutrition by the surrounding cells and is replaced by more or less excretory matter. Meanwhile the mass of cells is migrating toward the margin of the gonad. Arrived there, the nearly hollow cluster is given off into the body fluid where division of the component cells continues until the last generation of spermatogonia is formed. By a slight growth the cells are changed into the spermatocytes of the first order. The two customary divisions of the cells ensue and the spermatids are formed and then change to sperm. Meanwhile the spherical mass has changed its shape to the saucer-shaped or lenticular mass of the adult spermatophore.

It may seem an unwarrantable assumption that the ordinary spermatophores are the result of the segmentation of a single primary spermatocyte. The idea was suggested, rendered probable perhaps, by the development of the giant spermatogonia. It seems proven by the fact that all the cells in a given group manifest the same stage of division. It certainly presents a striking appearance, (figs. 8 and 9), to have a group of cells—a spherical bunch—all in the early prophase, for instance, when the adjacent cells manifest no sign of division. The possibility has

been considered that, perchance, some influence emanates from the central cell of a fortuitously accumulated mass as this cell divides, which, passed on to the adjacent cells, causes them to divide also. One is at a loss however to see why the influence should not be passed on to the still more peripheral cells so that all the cells of the gonad would divide more or less in unison.

It is proper to speak of the division of these cells as synchronous only in a general way. It must not be taken to mean that each phase of division occurs in all simultaneously, merely that certain prophase and telophase conditions, that anyway last for a considerable time, are frequently found in all or nearly all cells of the group at once, so that the cells of the group in some stage of division, not necessarily *exactly* the same, will contrast with the surrounding tissue.

One finds these synchronous cells more or less disconnected at times, as if the stress of the neighboring growing cells had broken the integrity of the mass. Still the general harmony of division is maintained, as would be the case if the blastomeres of a four- or eight-cell stage in a developing egg were separated by mechanical means. When this does occur the separated spermatogonial blastomeres apparently proceed to form spermatophores of a fourth or an eighth the normal size, thus readily accounting for the previously noted variation in the size of the spermatophores.

#### THE SPERMATOPHORE THE GAMETOOZON

It seems evident, then, that the development of the primary spermatogonia in the gonad, the same spermatogonia, when shed singly into the body cavity, and the giant spermatogonia are all in accord and are sufficiently suggestive of the development of the individual derived from the egg to make the hypothesis quite plausible at least that we have in *Arenicola* an alternation of generations. The primary spermatogonia are asexual spores, each of which, cleaving in a manner quite analogous to the cleavage of an egg, produces an individual, the spermatophore, all the cells of which are transformed into gametes.

## THE ALTERNATION OF GENERATIONS

There follows the union of the sperm and the egg, the second individual in the alternation, what we ordinarily know as the adult worm. This individual it is that produces the spermatogonia or oogonia, or in other words, the asexual spores.

*Botanic use of the term*

The conception of the alternation of generations has developed in its clear-cut simplicity among the botanists. It is that in the life history of a form there are two generations, one of which produces sexually, the other by asexual spores. It is typified in the bryophytes and most pteridophytes. In the higher plants the sexual or gametophyte generation is gradually reduced so that it is only in relatively recent times that its existence as such has been recognized in the phanogams.

*Usual zoological significance*

The term alternation of generations has been used by zoologists in a totally different sense. Two generations occur in many animals, the so-called sexual and asexual. The latter originates from a fertilized egg; the former arises by budding or a similar process from the asexual generation. There is thus an alternation of a generation that reproduces sexually with one that is never sexual, but the latter does not reproduce by asexual spores as is the case in plants. It is unfortunate that the same term is used for both processes. The asexual generation in the animal alternation is much more comparable to the sporophyte which is produced in propagation by cuttings or by runners. I am using the term alternation of generations strictly as it is understood by botanists.

## ALTERNATION OF GENERATIONS AND REDUCTION

Now in all except the lowly plants, in all the archegoniates and even in many algae this alternation of generations is accompanied by the phenomenon of chromatin reduction, and reduction *seems*

always to occur with definite relation to the alternation. The gametophyte, the plant that gives rise to the sexual elements, bears the reduced or haploid number of chromosomes. The sporophyte, the generation that produces the asexual spores, has the diploid or somatic number. Reduction occurs at the time the asexual spores are produced. So generally is this true, that for a time in many botanical papers, the presence of the diploid number of chromosomes was looked upon as a criterion that the cell possessing this number belonged to the sporophyte; or is a gametophyte cell if it has the haploid number, and the conclusion reached in a study of the archegoniates is forced, on *a priori* grounds, to cover the thallophytes as well. Thus Yamanouchi speaking of Williams' work on Dictyota says "The fertilized egg nucleus gives rise to an asexual plant with double the number of chromosomes and *consequently* a sporophyte generation." (Bot. Gaz., vol. 42: p. 431). And again, quoting from Davis, "Morphologically we can distinguish sporophyte plasma from gametophyte plasma by the double number of the chromosomes." (Am. Nat., vol. 39: p. 456).

In subjecting this life history [of Coleochaete] one of the green algae to what is regarded as a critical test of the two generations it has been discovered that this special spore-producing body is not a sporophyte. The test has to do with the number of chromosomes in the nucleus, a number which is definite for each plant species. The chromosomes are doubled in number by the fusion of the sperm and egg to form the oospore; and this means that in some other point in the life cycle the number must be reduced again. Accordingly the sporophyte, which arises from the oospore, is characterized by the double or  $2x$  number of chromosomes in its nuclei; and the gametophyte, which gives rise to the gametes, is characterized by the reduced or  $x$  number of chromosomes. Text book of Botany, Coulter, Barnes, Cowles; vol. 1, p. 32.

### *Alternation and reduction independent*

Recently, however, cytological studies on botanical material have thrown serious doubt on this conception. Reduction and the alternation of generations are, even in plants, independent phenomena. I shall briefly cite three lines of evidence in proof of this proposition. The few papers to which I refer will give references to abundant literature.

1. It is proven by cytological studies on aposporous and apogamous material. By 'apospory' (Vines, *Journal of Bot.*, '78, p. 355) is meant the direct production of a gametophyte from the tissue of a sporophyte without the intervention of a spore. 'Apogamy' (DeBarry, *Bot. Zeit.*, '78, p. 449) means the growth of a sporophyte as a vegetative outgrowth from the gametophyte. This definition is tentative, as later writers, Strasburger, Farmer and Digby, etc., are not yet agreed on the limitations of apogamy and parthenogenesis.

Farmer and Digby succeeded, in four forms with which they experimented, in inducing apogamy, causing the omission of sporogenesis. They derived the prothallia directly from abortive sporangia or from pinnae. Such gametophytes have approximately the diploid instead of the usual haploid number of chromosomes. They conclude therefore "that there is no necessary relation between the periodic reduction in the number of chromosomes and the alternation of generations."

Again, Yamanouchi, in his study of apogamy in *Nephrodium*, obtained a sporophyte with the haploid instead of the customary diploid number of chromosomes.

2. The independence of the alternation of generations and reduction is further demonstrated by the fact that reduction may occur before, after, or during the sexual act, that is, in either the sporophyte or the gametophyte generation. I realize that in such a statement of the argument I am begging the question. I am merely stating the facts as they appear from my standpoint.

Whether the sporophyte and gametophyte of the archegoniates are phylogenetically continuous with the spore-bearing and gamete-bearing generations of the algae or whether the sporophyte of the archegoniates is a new structure, developed out of the fertilized egg and unrelated to the spore-bearing generation of the ancestral alga is a moot point. Botanists are far from agreed as to the course of the evolution of the higher plants from their algal ancestors. The Chlorophyceae has been designated the probable ancestral group and both *Chara* and *Coleochaete* are pointed out by different investigators as probable connecting links. With equal conviction other botanists, notably Schenk recently,

discard these forms as pathways of ascent and adopt the Phaeophyceae as the most likely progenitors of the higher plants. Still others believe some common ancestors of these groups, a form now extinct, to have been the starting point of the archegoniates. Disagreeing over the probable course of evolution, they are equally at variance on the moot point mentioned above.

Insuperable difficulties seem, so far, to stand in the way of tracing the evolution of the sporophyte of higher plants from the so-called rudimentary sporophyte which develops from the fertilized egg of such forms as *Oedogonium*, *Ulothrix* and *Coleochaete*. In these forms the egg, after fertilization, breaks up into a number of separate cells, each of which gives rise to a new plant, thus functioning in a way suggestively like the asexual spores of the archegoniates. In *Coleochaete*, the only one of the lot that approaches the Hepaticae in structure sufficiently to be considered a probable ancestor, this structure can not be considered a sporophyte unless it be one with the  $x$  number of chromosomes instead of the  $2x$ , in which case it is difficult to see how it gives rise to the sporophyte of the higher forms, which is usually characterized by the  $2x$  number.

In quite as many algae, *Sphaerella*, *Volvox*, *Vaucheria*, *Chara*, *Fucus*, *Dictyota*, etc., the fertilized egg, possibly after a rest period develops directly into the spore-bearing generation. It may be invidious for a zoologist to suggest that the sporophyte of the higher plants has arisen from this class of algae, by the inclusion of the spore-bearing generation of an alga within the gamete-bearing generation, somewhat as in *Volvox* one individual is included within the other. And I will not even venture the suggestion but will merely call attention to the fact that botanists are still not in sufficient agreement as to the course of the origin of the sporophyte in the archegoniates to prejudice, by their plant evidence, a zoologist against a theory along this line for animals. It is such a point of view that I take, namely, that in animals, and possibly also in plants, the spore-bearing and gamete-bearing generations of the protozoa (and algae) are phylogenetically continuous with the sporozoon (or sporophyte) and the gametozoon (or gametophyte) of the higher forms.

With such a position in mind, I have a right to take evidence on the independence of reduction phenomena and the alternation of generations from the algae and protozoa. Moreover I have the precedent set by eminent botanists who use *Coleochaete*, *Fucus*, *Polysiphonia*, etc., as examples of the alternation of generations in the algae and base their theories of the rise of the phenomenon on such algal evidence.

Karsten has shown that the mitoses in the zygote of *Spirogyra* are reduction mitoses. The same is true of the *Desmidiaceae*. These mitoses occur, of course, after the fusion of the egg and sperm. In *Fucus*, reduction occurs in the division of the antheridial and oogonial initials; in the case of the egg, the reduction is three mitoses prior to fertilization. (Yamanouchi.) In the *Dicetyotaceae*, the cells of the tetrasporangium are the seat of the reduction division (Williams). Yamanouchi has shown that in *Polysiphonia* it is in the division of the tetraspore mother-cells that reduction occurs, while Wolfe claims that in *Nemalion* reduction occurs at the time of carpospore formation. Davis is so impressed with the fact that among the algae reduction occurs at so many different phases of the life history that he concludes that the phenomenon has a multiple origin. He says:

All of these cells in being the seat of reduction mitoses are analogous to the spore mother cells of archegoniates, but that would not warrant their being considered homologous with the latter structures. There is, on the contrary, good reason to believe that, in plants, reduction phenomena became established as features in the life histories of a number of groups quite independently of one another, as the evidence indicates was also true of the processes of sexual evolution and the differentiation of the sporophyte generations. (*Am. Nat.* 43: 109).

Similarly among the protozoa we find the reduction phenomena occurring at various times. In *Adalia* (Siedlecki) and *Monas* (Prowazek) reduction occurs by the formation of chromidia in the gametes before they unite. Neresheimer and Metcalf find that in *Opalina* reduction occurs before the gametes form. Schaudinn has shown that in *Actinophrys* the cytoplasm of the conjugating individuals unites and then a reduction of the nuclei occurs.

Dangeard thinks that reduction occurs during germination in *Chlamydomonas*. Prowazek's work on *Polytoma* indicates that the same is true in this form. Illustrations might be multiplied. Hertwig, years ago, after careful study of many protozoa, concluded that either before, after or during the sexual union there is a reduction division.

3. Among the protozoa and algae already cited, it is evident that reduction occurs when there is no alternation of generations.

#### CHAMBERLAIN'S THEORY

I have at some length presented what seems to me good evidence of the proposition that the alternation of generations and reduction are independent phenomena. I have been anxious to make the matter emphatic, otherwise, in any comparison of the alternation of generations in the higher plants and animals, unwarranted conclusions are reached. Thus Chamberlain, in proposing a theory of the alternation of generations in animals says,

The egg with the three polar bodies constitutes a generation comparable with the female gametophyte in plants; similarly, the primary spermatocyte with the four spermatozoa constitute a generation comparable with the male gametophyte in plants. All other cells of the animal constitute a generation comparable with the sporophyte generation in plants, the fertilized egg being the first cell of this series.

In support of this theory I shall present two lines of evidence: (1) the gradual reduction of the gametophyte in plants, with the constantly diminishing interval between the reduction of the chromosomes and the process of fertilization; and (2) the phenomena of chromatin reduction in both animals and plants.

Briefly, his argument is this: that since, in the higher plants the gametophyte is gradually reduced, producing a condition apparently identical with that in animals, the egg and its three polar bodies and the spermatids of animals are to be regarded as tetraspores and the gametophyte generation is undeveloped except as represented by these cells: that these cells are further proven tetraspores because, in their formation, reduction occurs in a manner very like the reduction in the formation of the tetraspores of the higher plants.

*Objections to Chamberlain's theory*

The facts adduced in the formation of the spermatophore of *Arenicola* and the evidence cited above to show the independence of reduction and the alternation of generations lead me to doubt both the validity of his argument and the accuracy of his conclusion. We are concerned at present only with his argument.

The fertilized egg of a lily is the first cell of the sporophyte, whether it ever divides at all. Consequently, we regard the zygospore of *Ulothrix* or *Spirogyra* and the fertilized egg of *Vaucheria* or *Oedogonium* as sporophytic structures, even if the first division of the zygote should be meiotic, as seems probable. From such a simple beginning, we believe that the more complex sporophytes with more conspicuous alternation have been developed. (*Am. Nat.* 44: 603).

## LIMITS OF GAMETOPHYTE AND SPOROPHYTE

The gametophyte and sporophyte generations must be marked off, then, it seems to me, by some limits independent of the phenomena of reduction. A much safer means of definition is to return to simple conceptions and designate that generation the gametophyte which had its origin in the asexual spore and which terminates with the formation of the egg. The sporophyte generation begins with the fertilized egg and terminates with the formation of the asexual spore. Presumably all would agree that this means of distinction is the best; the only reason for relying on any other criterion is the difficulty of applying this one.

Yet to insist that the conclusion reached in the study of the archegoniates that the  $2x$  number of chromosomes marks the sporophyte, a law not without exceptions, even in the archegoniates themselves, shall apply in the algae, protozoa and higher animals too, seems to me unwarranted, for it drives one to the conclusion that as great a reduction has occurred in the gametophyte among protozoa and algae, *Fucus*, for instance, as has occurred in the whole evolution of the archegoniates from lowly *Hepaticae* to the most specialized angiosperms; and because there is another possibility, namely, that the reduction phenomenon, in the course of evolution, shifts its position with reference to the boundaries of

the gametophyte and sporophyte generations in the life history of plants and animals—a possibility that, to me, seems more plausible.

In algae and protozoa alike we have the usual asexual reproduction followed by the sexual method, when some change in the physical or chemical condition, either of environment or organism, is an active cause. This is apparent from Calkins' studies on the conjugation of *Paramoecium*, Kleb's work on the formation of the gametes in *Hydrodictyon* and similar papers that followed these pioneer investigations. In these forms, as in most algae and protozoa, there is an intercalation of sexual among asexual generations, rather than an alternation. But let those conditions which produce sexuality recur with rhythmic regularity and an alternation is developed such as we have in *Dictyota*. We may presume that, phylogenetically, the higher forms exhibiting the alternation of generations have arisen from those lower ones that possess an intercalation of the sexual among the asexual generations. Further discussion of the point may be deferred and the alternation in *Arenicola* presented more fully.

### *Graphic life histories*

In *Arenicola* the individual male is a sporozoon giving rise to certain cells, the spermatogonia, which are really the microspores. These cleave in a manner homologous with the cleavage of the egg and give rise to the gametozoon or the spermatophore whose development is a curtailed recapitulation of the primitive gametozooic generation. At the end of this generation reduction occurs during gametogenesis. The union of the gametes initiates the sporozoic generation again. This life history may be graphically represented by the conventional diagram I, (text fig. 3).

In the higher dioecious plants we should have a very similar graphic life history, see diagram II, except for the fact that reduction occurs at a different point. Comparing merely these two life histories, it seems difficult to homologize the generations, simply because so important a phenomenon occurs at such widely separate points. Not only does reduction occur at different phases of the life history of the higher plants and animals, but

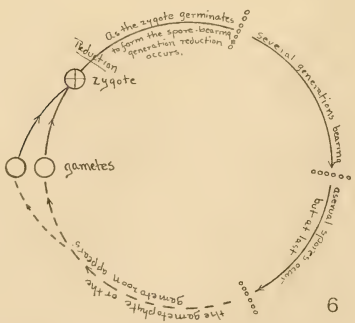
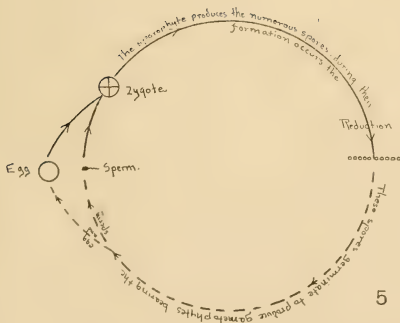
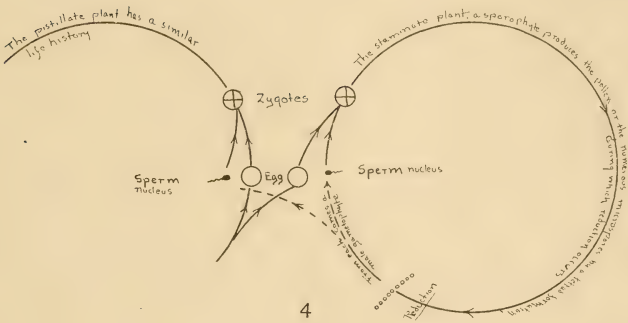
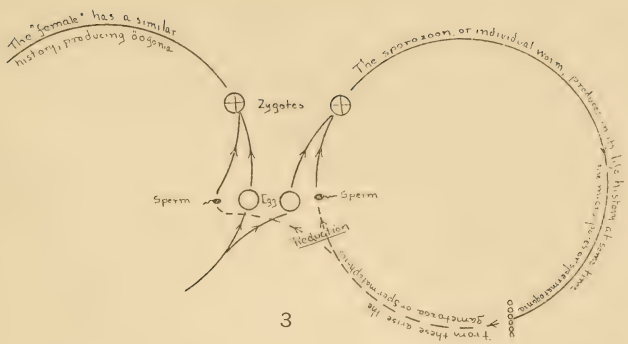


Fig. 3. Diagram I. Schematic representation of the spermatogenesis and alternation of generations in *Arenicola*.  
 Fig. 4. Diagram II. Scheme representing the life history of a phaenogam.  
 Fig. 5. Diagram III. Scheme of the life history of the moss or other bryophyte.  
 Fig. 6. Diagram IV. Scheme of the life history of such an alga as *Spirogyra* or such a protozoon as *Chlamydomonas*.

this is true, too, in the algae, as already pointed out. Let us review the facts down into that group with diagrams.

The mosses, (diagram III), show a sporophyte and gametophyte generation of about equal importance. The egg and sperm are usually borne on the same gametophyte.

The sporophyte becomes less and less prominent in the archegoniates as we approach the algae. Among the green algae the intercalation of a sexual generation seems the rule rather than the alternation of generations. The life history of *Spirogyra*, of the Diatomaceae, and of such protozoa as *Chlamydomonas* and *Poly-*

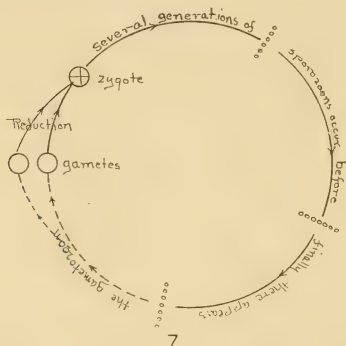


Fig. 7. Diagram V. Scheme of the life history of *Adelia*, *Monas*, etc.

toma would be graphically shown in diagram IV. In such forms as *Adelea*, *Monas* and *Actinophrys* among the protozoa, in no algae so far as I know, reduction occurs during the fusion of the gametes; the life history is given in diagram V (text fig. 7).

Now which of these life histories most nearly approximates primitive conditions? The question involves a discussion of the relation in phylogeny of the alternation of generations, sexuality and reduction. The evidence already given shows that the latter two phenomena antedate the alternation of generations, for both are found among simple animals and plants that have not achieved the alternation.

## SEXUALITY AND REDUCTION

Many biologists are inclined to regard sexuality and reduction as causally related. Thus Davis says "Chromosome reduction as a physiological process seems to be a corollary of sexual nuclear functions." (Bot. Gaz., vol. 43: p. 109.) If either were the effect of the other, we should expect reduction to bear a fixed relation to the sexual act. As it is we have seen that it may occur before, during or after such act. It would be better to say, then, that reduction is an adjunct of sexuality and both are probably corollaries of some fundamental cause yet to be determined.

It has been suggested by Bütschli and later writers that the union of the gametes is a process of rejuvenescence. In other words, the sexual act is induced by exhaustion of the organism. We know that chromidia are due to degeneration produced by exhaustion and otherwise (Dobell, '07). Further, a very simple type of reduction has been found in some protozoa (Monas, Adelea, etc.) to consist of the extrusion of chromidia. The very incomplete state of our knowledge regarding reduction, chromidia and sex, particularly among the lower plants and animals, makes the proposal of even a working hypothesis premature. I call attention to the exhaustion of the organism through repeated asexual divisions as a possible cause of both sexual union and reduction, merely to illustrate the possibility that both are corollaries of some underlying single cause.

About the only justifiable conclusion regarding the phylogenetic relation between the sexual act and reduction is that they arose as adjacent phenomena. This seems probable, since in primitive forms, both plants and animals, they occur close together in point of time. It would seem, on *a priori* grounds, too, that if a sexual union occurred in forms that had been producing asexually, a reduction in the amount of chromatin would promptly occur if it had not occurred previous to the act, so as to restore customary conditions as speedily as possible.

## THE PRIMITIVE ANIMAL TYPE

Botanists seem agreed that the significance of the persistent gametophyte in the higher plants is that it represents a return to a primitive type. The conclusion seems a proper one. We would similarly expect the gametozoic generation, then, to represent a primitive animal type. The spherical spermatophore, arising from a spermatogonium as a spore might cleave, must find its counterpart in the phylogeny of the higher animals as the thallus-like gametophyte of the higher plants indicates a thal-line ancestry for them.

Possibly the Volvocales approximate most closely to such an hypothetical ancestral form. The group is one that has been appropriated by many zoologists as showing marked animal affinities, and is admittedly a widely aberrant type by all botanists. *Volvox* reproduces asexually; certain cells of the central cavity, the so-called parthenogonidia, reproduce by fission, and the offspring divide and subdivide, the products cohering in spherical masses to form colonies like the parent. These are freed when the parent colony disintegrates. *Volvox* also reproduces sexually. Certain cells differentiate as eggs and come to lie in the interior of the colony. Other cells, similarly discharged to the interior, develop within them numerous sperm. The sperm break out of their containing cells and fertilize the eggs. The oospore remains inactive for some time as a resting spore but finally develops a colony like the parent. My preparations are not yet sufficiently clear to permit of working out all the chromatin changes, but I am quite positive that reduction occurs in *Volvox*, as it does in animals, during gametogenesis.

In the Dicyemidae, looked upon by many zoologists as the connecting link between the protozoa and the metazoa, the process of reproduction is suggestively similar, except that the asexually produced young bore out from the parent to an independent existence instead of awaiting the death of the parent. The center of the dicyemid is occupied by a cell, in which, besides the nucleus, there are one to several embryo cells from which the asexual individuals arise. These cells are evidently the homologues of

those parthenogonidia found in the *Volvox* colony. Max Hartmann, in describing the reproduction of the Dicyemidae, speaks of this central cell as the agametangium because in it develop the asexual, or as he calls them, the 'agamic' individuals. After several generations of these agamic individuals, there arise the sexual or the gametic individuals. They arise from the embryo cells (Keimzellen of Hartmann) by a process of cleavage very similar to the development of the agamic individuals, except that in the female the reproductive cells are split off early. The female as well as the male sexual individual grows within the asexual parent. The eggs are developed from the embryo cells of the female. They are freed from the gametic individual into the so-called agametangium of the agamic individual by the death of the gametic form. The egg matures by forming polar bodies and is fertilized by sperm which have discharged from the male gametic individual by its death and disintegration.

To harmonize Hartmann's description with the language I have used in giving the alternation of generations in the Arenicolidae I have only to change his terms slightly; (I have adopted the terminology proposed by Beard, cited later). Call his agamic individuals the sporozoon and the Keimzellen spores; his gametic individual, the gametozoon; then the dicyemids make concrete our conception of how the alternation of generations of the higher forms arose from such simple ones as *Volvox*, even though the line of ascent may not actually have passed through the Dicyemidae.

Imagine that in a *Volvox*-like form the sexual colony or gametozoon develops eggs and sperm before it is discharged from the colony which reproduces asexually and the condition of the dicyemids is practically achieved. Now imagine that a regular alternation of generations is established instead of the intercalation of an occasional sexual generation in the midst of the dominant asexual reproduction of the Dicyemidae and a condition is established that needs little if any modification to give the alternation of generations as we find it in the Arenicolidae, as follows:

The adult *Arenicola*, a sporozoon, corresponds to the sporophyte colony of *Volvox* or the agamic individual of the dicyemid. At

some time in its life history cells are developed within its central cavity—the primitive germ cells or spore mother cells. These develop spores which we know as spermatogonia or oogonia. Such spores, in *Volvox*, develop new colonies which may be sexual and which are freed when the parent disintegrates. In the *Dicymidae*, if a gametic individual develop from such spores (or Keimzellen) it is retained in the parent where it disintegrates to free its egg or sperm. In a word the gametozoon degenerates prematurely within the sporozoon. Now in *Arenicola*, I take it, the development of the spermatogonia into the spermatophores is the development of the gametozoa. Gametozoon degeneration begins before its development is complete and the sperm are produced by a short cut. Instead of developing an individual, within which some cells form the clusters of sperm, its cells form the sperm cluster immediately. In this genus, as I have already shown is the case in *Hydra*, the sperm seems to form within the spermatid, reminiscent perhaps of the primitive condition found in *Volvox* of forming the sperm within the cell.

For the sake of my theory, I should like to agree with Tannreuther in the spermatogenesis of *Hydra*. He claims that the spermatozoa develop in groups, each group enclosed within a single cell or cyst. But the clearness of my own preparations seems to negative completely such an interpretation. I can only reiterate what I have already published on the spermatogenesis of this form. It is too bad, for *Hydra* would make even a better transition form than it does, between such a spermatogenesis as we have in *Volvox* and the typical animal spermatogenesis in which the spermatid transforms into the sperm in its entirety rather than developing the sperm within it.

Hartmann, in the paper on the *Dicymids*, describes briefly and figures a development of the fertilized egg that is very similar to the development of the asexual spores. The same parallelism is noted in many botanic papers, the oospore developing much as an asexual spore does in its early stages. To find, then, in *Arenicola* that the spermatogonia or asexual spores develop to produce the spermatophore or gametozoon in a manner homologous to the development of the fertilized egg is to strengthen the position taken that the spermatophore is the gametozoon.

THE GAMETOZOON, A  $2x$  FORM

Botanists, reasoning on the basis of the fact that the disappearing gametophyte in the higher forms possesses the haploid number of chromosomes, have been led to assume, not only that the gametophyte represents a reversion to the primitive type, but also that this primitive plant possessed the  $x$  number of chromosomes. Reasoning in an analogous manner, we should be forced to assume, that, since the gametozoon possesses for most of its life history, the  $2x$  number of chromosomes, so the primitive animal type did not have the reduced number. This distinction between plants and animals has long been recognized. "So far as groups of plants above the thallophytes are concerned, the period of chromosome reduction has been found to be always associated with sporogenesis and never with gametogenesis as in the case of animals." (Yamanouchi; *Polysiphonia*, p. 43.) The difference may help to trace the gradual separation of the plant and animal types in the course of evolution. Upon this distinction, for instance, Dobell rests his belief in the plant affinities of the *Phytomonadina* (The structure and life-history of *Copromonas subtilis*, p. 112). The discovery already mentioned that *Volvox* has reduction occurring during gametogenesis would justify, in a measure, the classification of the form as an animal rather than as a plant.

## THE COMMON PROTOTYPE

Presumably plants and animals have come from a common ancestor. Now in all higher animals reduction occurs near the close of the gametozoic generation. It occurs, in all higher plants, near the close of the sporophyte generation. In the common ancestor it must have occurred at a point between these two extremes, possibly during or in close connection with the conjugation of the gametes. Such a possibility is rendered probable from the fact that, in thallophytes and protozoa, the reduction occurs at variable times in the life histories, usually as an adjunct of the union of the gametes, as if that variation were dominant which later becomes fixed in the two prevailing plant and animal types.

## ORIGINAL POSITION OF REDUCTION

If reduction originally occurred in the primitive common ancestor somewhere closely adjacent to the union of the gametes, the reduced number of chromosomes would exist for only a short period and might not occur at all. The major part of the life history of the forms would possess the somatic or diploid number. This is now the case for most animals and for many of the algae as already shown. In plants, then, the place of reduction in the life history has been shifted; the phenomenon has been postponed. In animals it occurs much nearer its original position.

*Reduction shifted*

Since in practically all animals and in many algae, the phenomenon of reduction occurs before or during conjugation of the gametes, the preponderance of evidence appears in favor of such a position in the primitive common ancestors of plants and animals. It is all the animals and many algae against the higher plants in favor of such an hypothesis. Text fig. 7, then, might nearly represent primitive conditions. Evidently following the gamete-bearing generation with its definite number of chromosomes would come a spore-bearing generation with the same number of chromosomes. This is true now in the Conjugales, Coleochaete, etc., and I believe in Volvocales and the animals. True, in Conjugales, Volvocales, etc., there are several spore-bearing generations following each other in succession. But if, for any reason, the alternation of generations be established by the omission of all but one of these spore-bearing generations, there would be left the gametophyte and sporophyte generations as I conceive them to exist in *Areuicola*, only that the reduction has shifted from a position like that of fig. 7 of the text to a place before the conjugation of the gametes. For plants the shift in position has been in the opposite direction—a shift that is seen progressing in text fig. 6 and completed in fig. 5.

In Chamberlain's theory of the alternation of generations in animals, he maintains that the shift in the animal group has

been in the same direction as in the higher plants. As pointed out by Coulter and Miss Pace, the end result attained in plants by the gradual reduction of the gametophyte generation to the point of complete extermination, in such forms as *Pandanus*, is entirely similar to the condition found in animals. Because the end results are similar is not *prima facie* evidence that the means of achievement in the course of evolution have been the same; it is very evident that in the higher plants such a condition as is found in *Cypripedium*, etc., is the result of a reduction of a gametophyte generation with the  $x$  number of chromosomes, for all steps in the process are evidently traceable. But nowhere in the animal kingdom, not even among the protozoa, is there any evidence of a corresponding gametozoic generation with a reduced number of chromosomes.

#### REDUCTION AND TETRAD-FORMATION

It is true that there is a striking similarity between the formation of the tetraspores in most plants and the development of the spermatids from the spermatocyte. It is rendered doubly suggestive by the fact that reduction occurs during both processes. Yet, even in the plants themselves, we are not warranted in concluding that all cells in which reduction occurs are homologous. Reduction occurs without tetraspore formation in a sufficient number of cases, as in *Lemanea*, *Chantransia*, etc., to show that there is no fundamental phylogenetic association involved. The customary appearance of a fourfold division at time of reduction may be based on some fundamental property of carbon compounds, possibly on the tetravalent condition of carbon itself; in which case it would not be strange to find tetrad formation common in plants and animals without assuming that, when occurring, a morphological homology is indicated.

#### BEARD'S HYPOTHESIS

In the preceding pages I have noted one hypothesis of the alternation of generations in animals—that proposed by Chamberlain, and I have given my reasons for discarding it. Another

hypothesis of the alternation of generations in animals has been proposed and vigorously advocated by Beard. He recognizes an antithetic alternation of generations in animals. He, too, identifies "the primary germ cells as the equivalents of the spore-mother-cells of plants." He regards the larva or phorozoon as the asexual generation, the homologue of the sporophyte. He derives the gametozoon, the adult animal, from it by apospory. He is forced to conclude, then, "that the final reduction of chromosomes has been deferred to a later portion of the life cycle in metazoa as compared with plants."

The same objections apply to Beard's hypothesis as to Chamberlain's, namely, that there is no evidence in fact of the successive steps in the postponement of reduction in animals similar to that so constantly apparent in plants. So far as we know, the process always occurs closely adjacent to the union of the gametes, continuing, in the higher animals, in much the same position in the life cycle that it occupies in the lower animals and plants. Whereas, in plants, it is evidently shifted from this primitive position and the successive steps of the shift are traced with some degree of certainty in living forms.

Furthermore, it seems to me, an impossible task to articulate his theory with what we know of the reduction phenomena and the development of the protozoa and mesozoa. He says:

The sexual generation of plants is at best a miserable failure from the morphological point of view. . . . The higher one ascends the smaller it becomes until in the higher plants it has almost reached the vanishing point, without, however, being able to disappear entirely.

In the animal it is the larva, the phorozoon, or asexual generation which makes the bravest show in the lower metazoa; . . . In the higher forms it becomes reduced.

But how will a relation be established between the larva of the metazoa and the asexual generation of the protozoa? The one should pass over into the other. It seems unwise to adopt a theory which demands a hiatus at this point, when it is easy to blaze a possible, uninterrupted trail along which evolution may have proceeded by way of such forms as the Volvocales and the Dictyemidae, if we adopt the hypothesis I have proposed.

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## PLATE 1

### EXPLANATION OF FIGURES

- 1 Latero-dorsal view of the second left nephridium of *A. cristata*.  $\times 15$ .
- 2 Latero-ventral view of the second left nephridium of *A. cristata*.  $\times 15$ .
- 3 Latero-dorsal view of the third left nephridium of *A. grubii*.  $\times 15$ .
- 4 Latero-ventral view of the fourth left nephridium of *A. marina*.  $\times 15$ .
- 5 Latero-dorsal view of the third left nephridium of *A. clapedii*.  $\times 15$ .
- 6 Latero-ventral view of the third left nephridium of *A. caudata*.  $\times 15$ . The bladder end shows a latero-dorsal view, but the rest of the nephridium is twisted by the weight of the gonad.



## PLATE 2

### EXPLANATION OF FIGURES

7 Section through the testis of *A. cristata* in October. It corresponds to the dotted portion of text fig. 1. The cells are some  $7.5\ \mu$  in diameter.

8 Section through a testis of *A. cristata* in February. It corresponds to the dotted portion of text fig. 2. Fibrous degeneration and phagocytosis are apparent.

9 Section through the testis of *A. cristata*; numerous spermatophores are forming.



### PLATE 3

#### EXPLANATION OF FIGURES

10 A spermatophore from the body fluid of *A. cristata*. These cells are the sixth generation of spermatogonia from the primary spermatogonia. Each divides to form the last spermatogonial generation. The cells have an average diameter of  $3.5\mu$ . All are in the equatorial plate stage.  $\times 340$ .

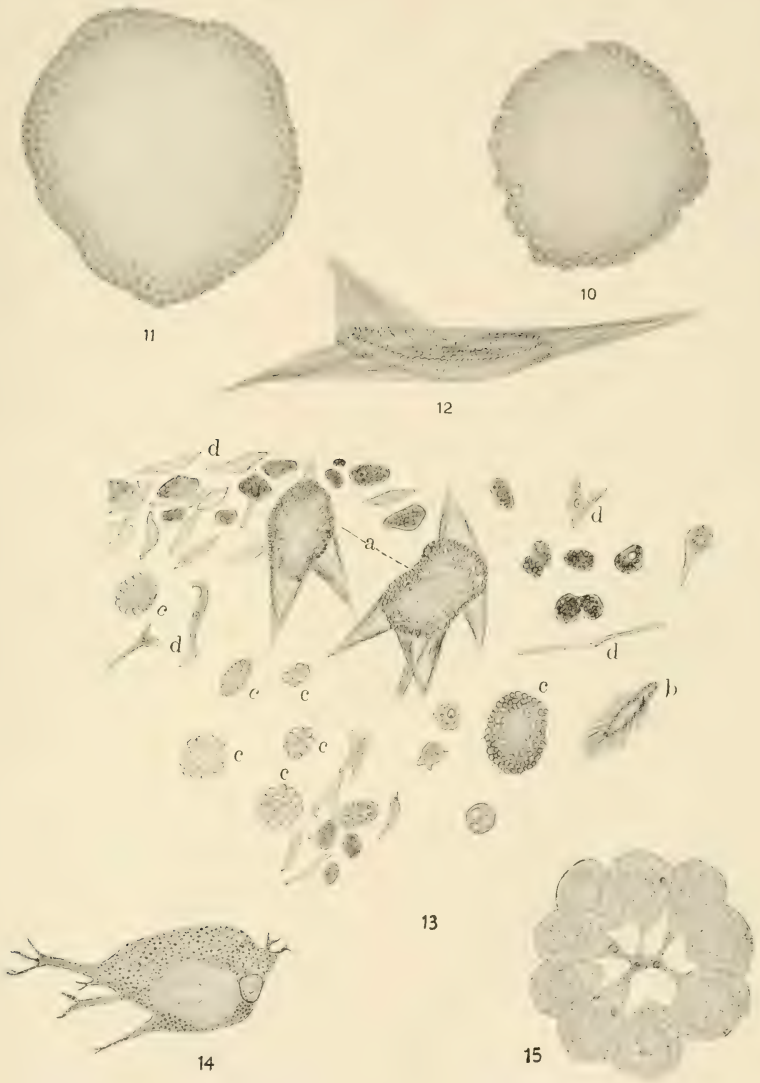
11 A spermatophore from the body fluid of *A. cristata*. The cells are the spermatids just after the division of the spermatocytes of the second division. They are in the early anaphase.  $\times 340$ .

12 A mature spermatophore of *A. cristata*. Smear preparation fixed in vom Rath's fluid and stained in saureviolett and iron haematoxylin.  $\times 625$ .

13 Body fluid of *A. cristata* under low power, showing spermatophores in various stages (a, b, c), and coelomic cells (d), leucocytes and chloragogue cells.  $\times 100$ .

14 A phagocyte from testis of *A. cristata* in April.  $\times 930$ . Note the ingested cell.

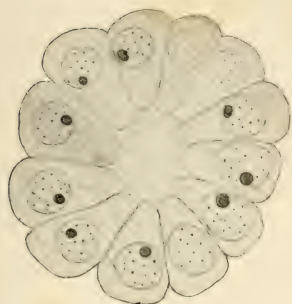
15 An early stage in the formation of a spermatophore, from the body fluid of *A. cristata*. Diameter of the spermatophore  $32\mu$ ; average diameter of the cells  $7.5$ .



## PLATE 4

### EXPLANATION OF FIGURES

- 16 Section of a more typical spermatophore.  $\times 1250$ .
- 17 A giant spermatogonium.  $\times 1800$ .
- 18 A giant spermatogonium dividing, the two-cell stage.
- 19 A giant spermatogonium dividing, the four-cell stage, polar view.
- 20 A giant spermatogonium dividing, the eight-cell stage, side view.
- 21 A giant spermatogonium dividing, the eight-cell stage, polar view.
- 22 A giant spermatogonium dividing, the sixteen-cell stage, polar view.
- 23 A later stage in segmentation of a spermatogonium, a spermatophore from the body fluid of *A. cristata*.
- 24 The 'invagination' of the forming spermatophore in *A. cristata*.
- 25 Section through the saucer-shaped spermatophore of *A. cristata*. The cells are spermatids,  $1\frac{1}{4} \times 4\mu$ .



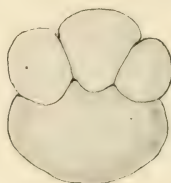
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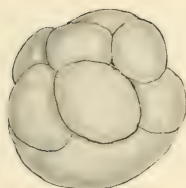
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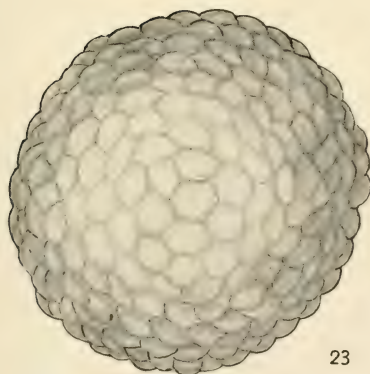
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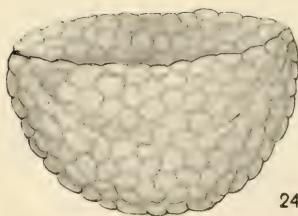
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22



23



24



25



# SUBJECT AND AUTHOR INDEX

- ALLEN, BENNET M.** The origin of the sex-cells of *Amla* and *Lepidosteus*. 1
- Amla** and *Lepidosteus*. The origin of the sex-cells of 1
- Ampelophila**. The effects of inbreeding and selection on the fertility, vigor and sex-ratio of *Drosophila*. 123
- Anatomical illustration before *Vesalius*. 945
- ANDREWS, E. A.** Male organs for sperm-transfer in the crayfish, *Cambarus affinis*; their structure and use. 239
- Animal geography. Physiological 551
- Ant-colony as an organism. The 307
- Arenicola** and a theory of the alternation of generations in animals. The formation of the spermatophore in 1001
- Armadillo quadruplets: a study of blastogenic variation. The limits of hereditary control in 855
- Asymmetry in the development of the serpulid, *Hydroides dianthus*. Experiments on the control of 927
- BIOGRAPHY:** Charles Otis Whitman xv
- Birds. On the olfactory organs and the sense of smell in 619
- Blastic variation. The limits of hereditary control in armadillo quadruplets: a study of 855
- CAMBARUS affinis**; their structure and use. Male organs for sperm-transfer in the crayfish 239
- Cell-division. The action of salt solutions followed by hypertonic sea-water on unfertilized sea-urchin eggs and the rôle of membranes in mitosis. The physiology of 695
- Changes in the relative weight of the central nervous system of the leopard frog. On the regular seasonal 663
- Chemistry of the white and yellow yolk of ova. On the formation, significance and 455
- Chickens. The transplantation of ovaries in 111
- CHILD, C. M.** The regulatory processes in organisms. 171
- Chromosomes. A review of the chromosomes of *Nezara*; with some more general considerations. Studies on 71
- Coelenterate ontogeny. Some problems of 493
- Control in armadillo quadruplets: a study of blastogenic variation. The limits of hereditary 855
- Control of asymmetry in the development of the serpulid, *Hydroides dianthus*. Experiments on the 927
- CRAIG, WALLACE.** Oviposition induced by the male in pigeons. 299
- Crayfish, *Cambarus affinis*; their structure and use. Male organs for sperm-transfer in the 239
- CURTIS, WINTERTON C.** The life history of the *Scolex* polymorphus of the Woods Hole region 821
- Cyclosalpa affinis* (Chamisso). The growth and differentiation of the chain of 395
- DAVENPORT, C. B.** The transplantation of ovaries in chickens. 111
- Differentiation of the chain of *Cyclosalpa affinis* (Chamisso). The growth and 395
- DONALDSON, HENRY H.** On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog. 663
- DOWNING, ELLIOT ROWLAND.** The formation of the spermatophore in *Arenicola* and a theory of the alternation of generations in animals. 1001
- DREW, GILMAN A.** Sexual activities of the squid, *Loligo pealii* (Les). 327
- Drosophila ampelophila*. The effects of inbreeding and selection on the fertility, vigor and sex-ratio of 123
- E**GGs and the rôle of membranes in mitosis. The action of salt solutions followed by hypertonic sea-water on unfertilized sea-urchin. 695
- Euschistus*. The spermatogenesis of 731
- F**ERTILIZATION in *Nereis*. Studies of 361
- Frog. On the regular seasonal changes in the relative weight of the central nervous system of the leopard 663
- G**ASTROPODS. The mechanism of locomotion in 155
- Geography. Physiological animal 551
- Geotropism of *Paramoecium*. The 993
- Growth and differentiation of the chain of *Cyclosalpa affinis* (Chamisso). 395
- Guinea pig. The cyclic changes in the ovary of the 37
- H**ARGITT, CHARLES W. Some problems of coelenterate ontogeny. 493
- HARPER, E. H.** The geotropism of *Paramoecium* 993
- Hemipteron, *Euschistus*. The spermatogenesis of an 731
- Hereditary control in armadillo quadruplets: a study of blastogenic variation. The limits of 855
- HOLMES, S. J.** Minimal size reduction in *Planarians* through successive regenerations. 989
- Hydroides dianthus*. Experiments on the control of asymmetry in the development of 927
- I**NBREEDING and selection on the fertility, vigor and sex ratio of *Drosophila ampelophila*. The effects of 123
- JOHNSON, MYRTLE E., W. E. RITTER and.** The growth and differentiation of the chain of *Cyclosalpa affinis* (Chamisso). 395
- L**EPIDOSTEUS. The origin of the sex-cells of *Amla* and 1
- LILLIE, FRANK R.** Biography: Charles Otis Whitman xv
- Studies of fertilization in *Nereis*, 361
- LILLIE, RALPH S.** The physiology of cell-division. IV. The action of salt solutions followed by hypertonic sea-water on unfertilized sea-urchin eggs and the rôle of membranes in mitosis 695
- Locomotion in *Gastropods*. The mechanism of 155
- LOCY, WILLIAM A.** Anatomical illustration before *Vesalius* 945
- LOEB, LEO.** The cyclic changes in the ovary of the guinea pig 37
- Loligo pealii* (Les). Sexual activities of the squid 327

- MEMBRANES** in mitosis. The action of salt solutions followed by hypertonic sea-water on unfertilized sea-urchin eggs and the rôle of 695
- Mitosis.** The action of salt solutions followed by hypertonic sea-water on unfertilized sea-urchin eggs and the rôle of membranes in 695
- MOENKHAUS W. J.** The effects of inbreeding and selection on the fertility, vigor and sex-ratio of *Drosophila ampelophila*. 123
- MONTGOMERY, JR., THOS. H.** The spermatogenesis of an hemipteron *Euschistus* 731
- NEREIS.** Studies of fertilization in 361
- Nervous system of the leopard frog. On the regular seasonal changes in the relative weight of the central 663
- NEWMAN, H. H., and J. THOMAS PATTERSON.** The limits of hereditary control in armadillo quadruplets: a study of blastogenic variation 855
- Nezara; with some more general considerations. A review of the chromosomes of 71
- OLFACTORY** organs and the sense of smell in birds. 619
- Ontogeny. Some problems of coelenterate 493
- Organs for sperm-transfer in the crayfish, *Cambarus affinis*; their structure and use. 239
- Ova. On the formation, significance and chemistry of the white and yellow yolk of 455
- Ovaries in chickens. The transplantation of 111
- Ovary of the guinea pig. The cyclic changes in the 37
- Oviposition induced by the male in pigeons. 299
- PARKER, G. H.** The mechanism of locomotion in gastropods 155
- PATTERSON, J. THOMAS, H. H. NEWMAN and.** The limits of hereditary control in armadillo quadruplets: a study in blastogenic variation 855
- Paramecium aurelia* and *Paramecium caudatum* 223
- Paramoecium*. The geotropism of 993
- Physiological animal geography. 551
- Physiology of cell-division. IV. The action of salt solutions followed by hypertonic sea-water on unfertilized sea-urchin eggs and the rôle of membranes in mitosis. The 695
- Pigeons. Oviposition induced by the male in 299
- Planarians through successive regenerations. Minimal size reduction in 989
- REGENERATIONS.** Minimal size reduction in Planarians through successive 989
- Regulatory processes in organisms. 171
- RIDDLE, OSCAR.** On the formation, significance and chemistry of the white and yellow yolk of ova 455
- RITTER, W. E., and MYRTLE E. JOHNSON.** The growth and differentiation of the chain of *Cyclosalpa affinis* (Chamisso). 395
- SCOLEX** polymorphus of the Woods Hole region. The life history of the 821
- Sea-urchin eggs and the rôle of membranes in mitosis. The action of salt solutions followed by hypertonic sea-water on unfertilized 695
- Selection on the fertility, vigor and sex-ratio of *Drosophila ampelophila*. The effects of inbreeding and 123
- Sense of smell in birds. On the olfactory organs and the 619
- Serpulid, *Hydroides dianthus*. Experiments on the control of asymmetry in the development of the 927
- Sex-cells of *Amia* and *Lepidosteus*. The origin of the 1
- Sex ratio of *Drosophila ampelophila*. The effects of inbreeding and selection on the fertility, vigor and 123
- Sexual activities of the squid, *Loligo pealii* (Les). 327
- SHELFORD, VICTOR E.** Physiological animal geography. 551
- Size reduction in planarians through successive regenerations. Minimal 989
- Spermatogenesis of an hemipteron, *Euschistus*. The 731
- Spermatophore in *Arenicola* and a theory of the alternation of generations in animals. The formation of the 1001
- Sperm-transfer in the crayfish, *Cambarus affinis*; their structure and use. Male organs for 239
- Squid, *Loligo pealii* (Les). Sexual activities of the 327
- STRONG, R. M.** On the olfactory organs and the sense of smell in birds. 619
- TRANSPLANTATION** of ovaries in chickens. 111
- VARIATION.** The limits of hereditary control in armadillo quadruplets: a study of blastogenic 855
- Vesalius*. Anatomical illustration before 945
- WEIGHT** of the central nervous system of the leopard frog. On the regular seasonal changes in the relative 663
- WHEELER WILLIAM MORTON.** The ant-colony as an organism. 307
- Whitman, Charles Otis: Biography. xv
- WILSON, EDMUND B.** Studies on chromosomes. VII. A review of the chromosomes of *Nezara*; with some more general considerations. 71
- WOODRUFF, LORANDE LOSS.** *Paramecium aurelia* and *Paramecium caudatum*. 223
- YOLK** of ova. On the formation, significance and chemistry of the white and yellow 455
- ZELENY, CHARLES.** Experiments on the control of asymmetry in the development of the serpulid, *Hydroides dianthus*. 927











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